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Identification of Volatile Compounds and Fatty Acids in Hatay Virgin Olive Oil and Its Antioxidant, Anticholinergic, and Antidiabetic Effects

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ABSTRACT
Extra virgin olive oil is a functional food of considerable importance in both nutrition and health due to its rich chemical composition and diverse biological activities. In addition to its traditional use for therapeutic purposes, it also holds high gastronomic value owing to its characteristic aroma. In this study, the volatile compound profile of extra virgin olive oil obtained from the Hatay region was comprehensively characterized using gas chromatography–mass spectrometry coupled with solid-phase microextraction (GC–MS–SPME), while its fatty acid composition was determined by gas chromatography with flame ionization detection (GC–FID) using a 37-component FAME mixture standard. The analysis revealed that oleic acid, a key quality indicator specific to olive oil, was present within the limits reported in the literature. Both the volatile and fatty acid profiles confirmed the presence of characteristic olive oil components, demonstrating the authenticity of the sample. The antioxidant capacity of the olive oil was evaluated comparatively against reference antioxidants, including BHA, BHT, α-tocopherol, Trolox, and ascorbic acid. Furthermore, enzyme inhibition assays indicated that the olive oil exhibits notable biological activity. In particular, its antidiabetic activity was found to be higher than that of the standard compound, highlighting its potential therapeutic value. In conclusion, this study demonstrates that extra virgin olive oil is not only a nutritionally valuable lipid source but also a potent functional food with potential protective effects against metabolic and neurodegenerative diseases. These findings suggest that olive oil may be considered a supportive agent in the prevention and management of such diseases.

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Hatay Sızma Zeytin Yağının Uçucu Bileşenleri ile Yağ Asitlerinin Tespiti ve Antioksidan, Antikolinerjik ve Antidiyabetik Etkisi

ÖZET
Sızma zeytinyağı, zengin kimyasal kompozisyonu ve çok yönlü biyolojik etkileri nedeniyle hem beslenme hem de sağlık alanında önemli bir yere sahip fonksiyonel bir gıdadır. Geleneksel olarak şifa amacıyla tüketilmesinin yanı sıra, karakteristik aroması sayesinde gastronomik açıdan da yüksek değere sahiptir. Bu çalışmada, Hatay yöresinden temin edilen sızma zeytinyağının uçucu bileşen profili gaz kromatografisi–kütle spektrometresi ile katı faz mikroekstraksiyon (GC-MS-SPME) tekniği kullanılarak detaylı şekilde karakterize edilmiş, yağ asidi kompozisyonu ise 37 bileşenli FAME karışım standardı ile gaz kromatografisi alev iyonizasyon dedektörü (GC-FID) aracılığıyla belirlenmiştir. Analiz sonuçlarında, zeytinyağı için karakteristik ve kalite göstergesi olan oleik asidin literatürde belirtilen sınırlar içerisinde yer aldığı saptanmıştır. Elde edilen hem uçucu bileşen hem de yağ asidi profili, örneğin zeytinyağına özgü karakteristik bileşenleri içerdiğini doğrulamış ve ürünün orjinalliğini ortaya koymuştur. Zeytinyağının antioksidan kapasitesi; BHA, BHT, α-tokoferol, Trolox ve askorbik asit gibi referans antioksidanlar ile karşılaştırmalı olarak değerlendirilmiştir. Bununla birlikte, enzim inhibisyon analizleri zeytinyağının dikkate değer düzeyde

Biyokimya

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Anahtar Kelimeler
Zeytin yağı
Uçucu bileşenler
Yağ asitleri
GC-MS-SPME-FID
Biyolojik aktivite

biyolojik aktivite sergilediğini göstermiştir. Özellikle antidiyabetik etkinliğin, kullanılan standart bileşenden daha yüksek bulunması, zeytinyağının potansiyel terapötik değerini ön plana çıkarmaktadır. Sonuç olarak, bu çalışma sızma zeytinyağının yalnızca besinsel açıdan değerli bir lipid kaynağı olmadığını, aynı zamanda metabolik ve nörodejeneratif hastalıklara karşı koruyucu etkiler sunabilecek güçlü bir fonksiyonel gıda olduğunu ortaya koymaktadır. Bu bulgular, zeytinyağının hastalıkların önlenmesi ve yönetiminde destekleyici bir ajan olarak değerlendirililebileceğini göstermektedir.

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INTRODUCTION

Extra-virgin olive oil (*Olea europaea* L.) is a widely used food in diets worldwide, especially in Mediterranean countries. It is considered one of the healthiest foods. Its consumption is increasing day by day, and the main reason for this is its proven health benefits (Gullon et al., 2020). Due to the bioactive components in olive oil, its cardioprotective, antioxidant, anti-diabetic, and metabolic effects have been reported through different mechanisms (Donat-Vargas et al., 2022; Martinez-Gonzalez et al., 2022).

The chemical composition of olive oil consists mainly of fatty acids in the form of triglycerides (98–99%); oleic acid, a monounsaturated fatty acid, is the most abundant of these. Other fatty acids, such as palmitic, stearic, linoleic, and α-linolenic acids, are also present in significant amounts. The high proportion of oleic acid contributes to olive oil's resistance to oxidation and its health benefits. This fatty acid profile has been particularly associated with a reduction in cardiovascular risk factors (Gonçalvez et al., 2024; Afonso et al., 2024; eFleyfeltel et al., 2022).

Olive oil also has a complex aroma profile containing volatile compounds (such as alcohols, esters, carbonyl compounds, and hydrocarbons). The composition of these volatile compounds can vary depending on the ripeness of the olive fruit, its variety, growing conditions, and processing methods. Volatile organic compounds determine the sensory characteristics of olive oil and also form a chemical signature that is important in distinguishing product quality (Melo Ferreira et al., 2025; Mascrez et al., 2024; Díaz-Montaña et al., 2023).

Antioxidants are pioneers in the treatment of many diseases due to their ability to eliminate oxidative stress and to prevent the formation of reactive species. (Gökkaya et al., 2026; İzol, 2025; Yurt et al., 2024; Çelik et al., 2024; Yılmaz et al., 2024). Olive oil has been reported to possess significant antioxidant capacity (Nakbi et al., 2010). Its antidiabetic potential is commonly evaluated *in vitro* by inhibiting α-glucosidase, while its anticholinergic activity is assessed by inhibiting acetylcholinesterase and butyrylcholinesterase (Yapıcı et al., 2025; Izol et al., 2025; Izol et al., 2026). Inhibiting these enzymes, which are implicated in metabolic diseases, using natural, side-effect-free components is highly significant.

In this study, the biological activities—namely antioxidant, anticholinergic, and antidiabetic properties—of extra virgin olive oil obtained from Hatay Province, one of Türkiye's major olive oil–producing regions, were evaluated. In addition, its chemical characterization was determined by analyzing fatty acid and volatile compound profiles using GC–MS.

MATERIAL and METHOD

Chemicals

The primary FAME-mix standard, enzyme and antioxidant standards (BHA, BHT, α-tocopherol, Trolox, and ascorbic acid), and all other chemicals (ABTS, $K_2S_2O_8$, AChE, BChE, DPPH, Folin–Ciocalteu reagent (FCR), trichloroacetic acid (TCA), Na_2HPO_4, NaH_2PO_4, $K_3Fe(CN)_6$, $FeCl_3$, CH_3COONH_4, CH_3COONa, CH_3COOK, $CuCl_2$, neocuproine, HCl, TPTZ, ethanol, Na_2CO_3, gallic acid, quercetin, $Al(NO_3)_3$, *p*-NPG) used in the analyses were obtained from Sigma Aldrich in analytical grade purity.

Preparation of olive oil for analysis

In enzyme inhibition studies, stocks prepared by dissolving olive oil in DMSO solvent were used. In antioxidant analyses, olive oil was dissolved in ethanol to prepare a stock solution, and analyses were performed using this stock solution.

Fatty acids by GC-MS-FID

For fatty acid analysis, olive oil was first esterified. For this purpose, 200 µl of olive oil was added to a test tube, followed by 6 mL of hexane, and finally 200 µl of 2N KOH solution. After centrifuging at 3600 rpm for 5 minutes, the supernatant was filtered through a 0.22 µm filter and transferred to a GC vial. The FAME-Mix Standard was used; no internal standard was used. The instrument and analysis conditions are given in Tables 1-2

Table 1. GC Properties for fatty acid analysis
Çizelge 1. Yağ asidi analizi için GC özellikleri

Parameters	Specifications
Gas Chromatograph	PerkinElmer Clarus 690
Oven Program	Cryogenics : Off Initial Temp : 120°C Initial Hold : 1.00 min Total Run Time : 35.50 min Maximum Temp : 250°C Equilibration Time : 1.0 min Ramp 1 : 10.0 0/min to 175°, hold for 5.00 min Ramp 2 : 5.0 0/min to 210°, hold for 5.00 min Ramp 3 : 5.0 0/min to 230°, hold for 8.00 min
Column	100 m, 0.25 mm, 0.25 µm film, Fused silica capillary column
Autosampler Method	Syringe Capacity : 5.0 µL Injection Speed : Normal Viscosity Delay : 0 Pre-injection Solvent Washes : 1 Post-injection Solvent Washes (A) : 2 Injection Volume : 1.0 µL Sample Pumps : 6 Wash/Waste Vial Set : 1 Pre-injection Sample Washes : 2
Carrier Parameters	Carrier A control : PFlow - He Column A length : 30.00 m Vacuum Compensation : ON Split Flow : 20.0 mL/min Initial Setpoint : 1.00 ML/MIN Diameter : 250 µm Initial Hold : 999.00 min Carrier B control : PFlow - He Column B length : 100.00 m Vacuum Compensation : OFF Split Flow : 50.0 mL/min Initial Setpoint : 2.00 ML/MIN Diameter : 250 µm Initial Hold : 999.00 min
Injector	B
Detector	FID
Software	Total Chrom ver 6.3.3

Table 2. MS Properties (İzol,2024)
Çizelge 2. MS Özellikleri

Parameters	Specifications
Mass Spectrometer	PerkinElmer Clarus SQ 8T
Scan Range	40 to 250 Daltons
Scan Time	0.2 s
Interscan Time	0.2 s
Inlet Line Temp	200 °C
Source Temp	180 °C
Multiplier	1700 V
Ionization	El+

Volatile compounds by GC-MS-SPME

For the analysis of volatile components, 3 mL virgin olive oil was transferred to a 20 mL GC vial, and after the vial was sealed, the SPME needle was immersed and heated at 50°C for 7 minutes, then injected into the device with the SPME needle. SPME characteristics are presented in Table 3, and GC analysis conditions for volatile analysis are presented in Table 4.

Table 3. SPME Properties
Çizelge 3. SPME Özellikleri

Parameters	Specifications
SPME	Supelco brand SPME Holder, Manual (Simaaldrich) 75 µm CAR/PDMS, Fused Silica, 24 Ga
Lot	117983

Table 4. GC Properties for aroma analysis
Çizelge 4. Aroma analizi için GC özellikleri

Parameters	Specifications
Gas Chromatograph	PerkinElmer Clarus 690
Oven Program	Cryogenics : Off Initial Temp : 70°C Initial Hold : 1.00 min Ramp 1 : 8.0 0/min to 120°, hold for 0.00 min Ramp 2 : 15.0 0/min to 220°, hold for 10.00 min Total Run Time : 23.92 min Maximum Temp : 230°C Equilibration Time : 2.0 min
Autosampler Method	Syringe Capacity : 5.0 µL Injection Speed : Normal Viscosity Delay : 0 Pre-injection Solvent Washes : 0 Post-injection Solvent Washes (A) : 2 Injection Volume : 1.0 µL Sample Pumps : 6 Wash/Waste Vial Set : 1 Pre-injection Sample Washes : 2
Column	30 m, 0.25 mm, 0.25 µm Elite-WAX
Carriers Parameters	Carrier A control : PFlow - He Column A length : 30.00 m Vacuum Compensation : ON Split Flow : 5.0 mL/min Initial Setpoint : 1.00 ML/MIN Diameter : 250 µm Initial Hold : 999.00 min Carrier B control : PFlow - He Column B length : 100.00 m Vacuum Compensation : OFF Split Ratio : 10.0 : 1 Initial Setpoint : 2.00 ML/MIN Diameter : 250 µm Initial Hold : 999.00 min
Injector	A
Software	TurbaMass Ver. 6.1.2
Library	NIST
Start time (min)	4.00
End time(min)	23.00

Antioxidant activity assays

Reduction assays

Apak and colleagues' approach (2006) served as the basis for the Cu^{2+} reduction assay. The absorbance was measured at 450 nm during a 30-minute incubation period at room temperature and in the dark after the sample was pipetted and shaken into test tubes at predefined concentrations.

A modified version of the Oyaizu method (1986) was used. At predetermined concentrations, the sample was pipetted into test tubes and vortexed. The absorbance of the sample at 700 nm was measured three times after the test tube was vortexed and left in the dark for 10 minutes following the addition of 1 mL of distilled water.

The approach utilized in Izol's research was implemented (2025). The FRAP reagent solution (2.5 mL, pH 3.6, 0.3 M) was made by mixing $FeCl_3$ (20 mM, 2.25 mL) with TPTZ solution (10 mM, 2.25 mL) in acetate buffer. After

dissolving the sample in 2.5 mL of buffer solution at varying concentrations (15–60 µg/mL), it was combined with 2.5 mL of 1% FRAP solution and incubated for 30 minutes at 37°C in the dark. At 593 nm, the sample's Fe^{3+}-TPTZ reduction capability was measured. For every concentration, the Fe^{3+}-TPTZ reduction capability was measured three times.

Radical scavenging assays

The Blois method (1958) was used to carry out the DPPH assay. Before use, the absorbance of the radical control solution and ethanol was set at 1.00±0.025 nm. After pipetting precise concentrations (15–60 µg/mL) into test tubes and vortexing them, the tubes were permitted to stand at room temperature and in the dark for 30 minutes. Their absorbance at 517 nm was compared with that of an ethanol-containing blank solution.

The ABTS assay used the methodology outlined by Re et al. (1999). Before use, the absorbance of the e control solution—which included phosphate buffer and ABTS+ solution—was set to 1.00±0.025 nm. The absorbances were measured at 734 nm after specific amounts of sample (5–20 µg/mL) were pipetted into test tubes containing 0.1 M phosphate buffer and 2 mM ABTS+ solution, vortexed, and incubated in the dark at room temperature for 30 minutes. The blank control was phosphate buffer.

Total phenolic and flavonoid content

In both methods, the sample concentration was adjusted to 1000 mg/kg. The Folin-Ciocalteu reagent (FCR) was used to measure the phenolic compound content of the sample. The prepared stock solution was used for the analysis. After dissolving 7 mg of gallic acid in 7 mL of distilled water, 100–1000 µL of the stock solution containing gallic acid was transferred to tubes, and the final volume was increased to 23 mL using distilled water. Then, under normal circumstances, 0.5 mL of FCR and 1.5 mL of 2% Na_2CO_3 were added and stirred for 2 hours. Ultimately, the absorbances were measured at 760 nm in comparison to a blank (distilled water). The sample was substituted with distilled water as a control. The standard was gallic acid. The samples' absorbances were computed as µg gallic acid equivalents (Singleton et al., 1999).

The total flavonoid content of sample was determined using the Park et al. method (1997). Quercetin was the typical flavonoid compound. First, equal volumes µL containing 10, 20, 30, 40, and 50 quercetin from the stock prepared for the graph were added to the tubes along with 0.1 mL (1.0 M) CH3COOK (in water) and 0.1 mL $Al(NO_3)_3$ (10%). 4.3 mL of ethanol was added after the liquid had been mixed and diluted. The mixture's absorbance at 415 nm was compared with that of a blank sample containing ethanol after a standard 40-minute incubation period. The total flavonoid content was calculated as µg quercetin equivalent using the equation derived from the standard quercetin graph (µg QE/g).

Enzyme inhibition assay

Acetylcholinesterase and butyrylcholinesterase inhibition assay

Enzyme activity was measured using DTNB, butyrylthiocholine, and acetylthiocholine as substrates. 5-thio-2-nitrobenzoic acid, a yellow compound that absorbs light at 412 nm, is created when thiocholine and DTNB mix (Ellman et al., 1961). Enzyme inhibition was measured using the IC_{50} value. In order to do this, a test tube was filled with buffer, distilled water, DTNB solution, enzyme solution, inhibitor, and substrate (acetylcholine iodide and butyrylcholine iodide) at predetermined quantities. The absorbance was then recorded at 412 nm for three minutes. Lastly, this equation was used to calculate the $IC_{5values}$ta after the relationship between enzyme activity (%) and concentration was established.

α-Glycosidase inhibition assay

The technique outlined by Tao et al. (2013) was used to determine the α-glycosidase inhibition ability. After adding buffer, purified water, enzyme solution, inhibitor, and *p*-NPG to the cuvette at predetermined quantities, absorbance was monitored for three minutes at 405 nm. Six distinct concentrations of bee product stock solutions were pipetted in order to get the IC_{50} value. IC_{50} values were computed after measuring absorbance at 405 nm for 3 min.

Statistical analysis

The findings of the triple antioxidant testing were shown together with their standard deviations. The SPSS program was used for statistical analysis, while Excel was used for further computations. Tukey's post hoc test was used for multiple comparisons, ANOVA was used to examine the data, and the significance threshold was set at $p < 0.05$.

RESULTS and DISCUSSION

Fatty acids

The fatty acids in olive oil were quantitatively determined using the FAME-Mix standard for 37 different fatty acids. The results for the fatty acids in olive oil are presented in Table 5, and the chromatogram is shown in Figure 1. The names and retention times of the standards are given in Table 6.

Table 5. Fatty acids in virgin olive oil
Çizelge 5. Sızma zeytinyağının yağ asitleri

Peak	Component Name	Time (min)	Area (uV*sec)	Area (%)
6	C11:0-Undecanoic acid	9.157	390.19	0.00
7	C14:0-Myristic acid	11.994	762.02	0.01
8	C15:0-Pentadecanoic acid	13.186	426.05	0.00
10	C16:0-Palmitic acid	14.605	1479311.54	14.70
12	C16:1-Palmitoleic acid	15.493	131396.99	1.31
13	C17:0-Heptadecanoic acid	15.869	13361.32	0.13
14	C17:1- cis-10-Heptadecenoic acid	16.789	21477.25	0.21
15	C18:0-Stearic acid	17.525	320517.26	3.19
16	C18:1-cis-Oleic acid	18.476	7194910.81	71.51
17	C18:2- cis-Linoleic acid	19.620	582016.55	5.78
18	C18:3 (n6)- ɣ-Linolenic acid	20.191	46671.96	0.46
19	C20:0-Arachidic acid	21.039	530.10	0.01
20	C18:3 (n3)- α-Linolenic acid	21.123	22783.98	0.23
21	C20:1- cis-11-Eicosenoic acid	21.206	58013.92	0.58
22	C21:0-Heneicosanoic acid	21.719	650.87	0.01
23	C22:0-Behenic acid	23.474	10820.07	0.11
24	C20:4-Arachidonic acid	25.254	1946.36	0.02
25	C23:0-Tricosanoic ac	25.718	167151.65	1.66
26	C22:2-cis-13,16-Docosadienoic acid	26.951	6773.33	0.07
27	C24:0-Lignoceric acid	28.159	1503.57	0.01
		Total	**10061415.81**	**100**

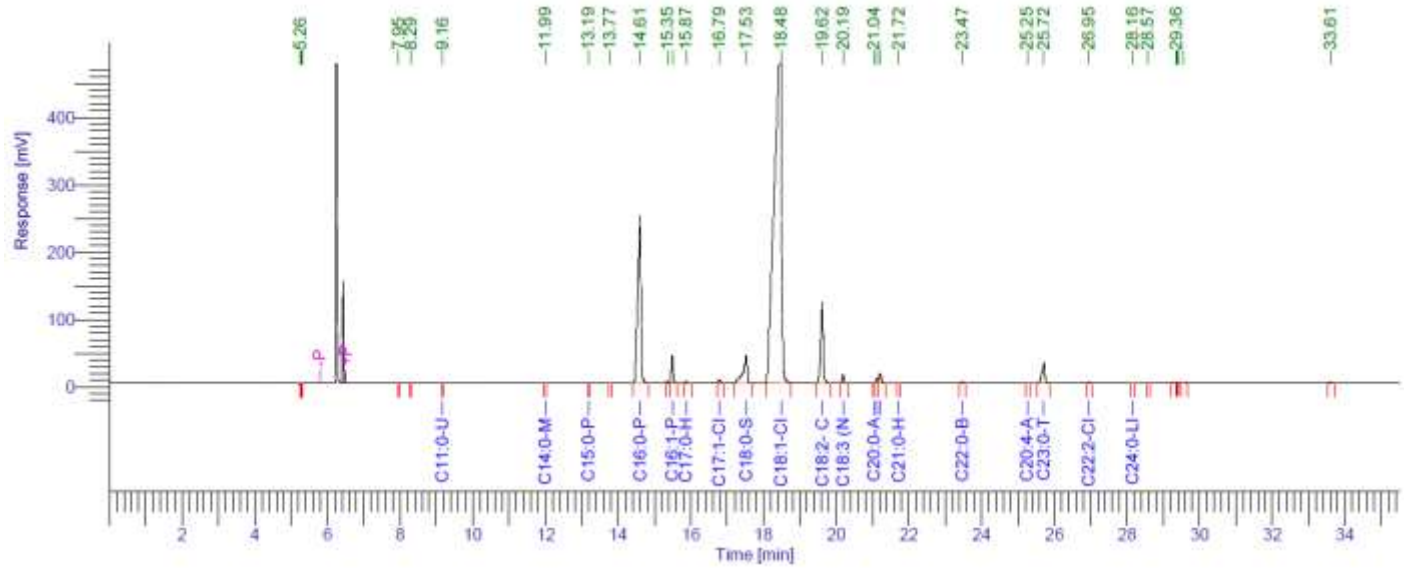

Figure 1. Fatty acids chromatogram of virgin olive oil
Şekil 1. Sızma zeytinyağının yağ asitleri kromatogramı

Olive oil differs from other vegetable oils in terms of its chemical composition and, in particular, its fatty acid profile, and its positive effects on human health are largely attributed to these components. This study identified a comprehensive fatty acid profile in olive oil, which includes saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA).

Oleic acid (C18:1 cis-oleic acid), identified as the predominant fatty acid in olive oil, typically accounts for 55–83% of total fatty acids and constitutes the characteristic biochemical property of olive oil. Numerous biological activities of oleic acid have been reported in the literature (Liu et al., 2023; Covas, 2007; Schwingshackl & Hoffmann, 2014). Furthermore, oleic acid extends the shelf life of olive oil by increasing its oxidative stability.

Table 6. FAME Mix standard 37-component fatty acids
Çizelge 6. FAME Mix standard 37 bileşenli yağ asitleri

Peak	Component Name	Time
1.	(C4:0) Butyric acid	6.554
2.	(C6:0) Caproic acid	6.927
3.	(C8:0) Caprylic acid	7.577
4.	(C10:0) Capric acid	8.604
5.	(C11:0) Undecanoic acid	9.275
6.	(C12:0) Lauric acid	10.066
7.	(C13:0) Tridecanoic acid	10.986
8.	(C14:0) Myristic acid	12.070
9.	(C14:1) Myrictoleic acid	13.073
10.	(C15:0) Pentadecanoic acid	13.262
11.	(C15:1) cis-10-Pentadecenoic acid	14.358
12.	(C16:0) Palmitic acid	14.572
13.	(C16:1) Palmitoleic acid	15.531
14.	(C17:0) Heptadecanoic acid	15.924
15.	(C17:1) cis-10-Heptadecenoic acid	16.915
16.	(C18:0) Stearic acid	17.331
17.	(C18:1n9c) Oleic acid	17.936
18.	(C18:1n9t) Elaidic acid	18.206
19.	(C18:2n6t) Linolelaidic acid	18.921
20.	(C18:2n6c) Linoleic acid	19.534
21.	(C18:3n6) ɣ-Linolenic acid	20.205
22.	(C20:0) Arachidic acid	20.601
23.	(C18:3n3) α-Linolenic acid	21.146
24.	(C20:1n9) cis-11-Eicosenoic acid	21.224
25.	(C21:0) Heneicosanoic acid	21.787
26.	(C20:2) cis-11,14-Eicosadienoic acid	22.731
27.	(C22:0) Behenic acid	23.588
28.	(C20:3n6) cis-8,11,14-Eicosatrienoic acid	24.008
29.	(C20:3n3) cis-11,14,17-Eicosatrienoic acid	24.708
30.	(C22:1n9) Erucic acid	24.968
31.	(C20:4n6) Arachidonic acid	25.350
32.	(C23:0) Tricosanoic acid	26.258
33.	(C22:2) cis-13,16-Docosadienoic acid	26.914
34.	(C20:5n3) cis-5,8,11,14,17-Eicosapentaenoic acid	27.052
35.	(C24:0) Lignoceric acid	27.989
36.	(C24:1n9) Nervonic acid	30.501
37.	(C22:6n3) cis-4,7,10,13,16,19-Docosahexaenoic acid	31.476

Palmitic acid (C16:0) and stearic acid (C18:0) are among the most common saturated fatty acids found in olive oil. Palmitic acid is generally found in the range of 7–20%, while stearic acid is found in lower proportions. It has been reported that stearic acid has a neutral effect on cardiovascular risk compared to other saturated fatty acids (Mensink and Katan, 1992). This explains why olive oil is considered a healthy fat source despite its saturated fat content.

Essential fatty acids such as linoleic acid (C18:2) and α-linolenic acid (C18:3 n-3) cannot be synthesized by the human body and must be obtained through diet. Linoleic acid plays an important role in cell membrane structure and eicosanoid synthesis, while α-linolenic acid stands out for its anti-inflammatory effects (Saffaryazdi et al., 2020; Simopoulos, 2002). Furthermore, ɣ-linolenic acid (C18:3 n-6) and arachidonic acid (C20:4), which are found in low amounts in olive oil, are important PUFA components involved in biological signaling pathways (Brash, 2001).

Monounsaturated fatty acids such as palmitoleic acid (C16:1) and eicosenoic acid (C20:1) support the metabolically active lipid profile of olive oil. It has been reported that palmitoleic acid may increase insulin sensitivity bactingly like lipokines (Long et al., 2014; Cao et al., 2008).

Components such as behenic acid (C22:0), lignoseric acid (C24:0), and tricosanoic acid (C23:0), which are long-

chain saturated fatty acids, are generally found in trace amounts and constitute the unique fingerprint of olive oil. These fatty acids are used as important indicators for determining the purity and origin of olive oil (Aparicio & Aparicio-Ruíz, 2000).

In conclusion, the fatty acid profile identified in this studl is consistent with the literature in its high oleic acid content, low saturated fat ratio, and biologically active PUFA components. This composition supports the positive effects of olive oil on cardiovascular health, anti-inflammatory effects, and metabolic balance.

Volatile compounds

The volatile components found in virgin olive oil are shown in Table 7. The GC-MS-SPME chromatogram is shown in Figure 2.

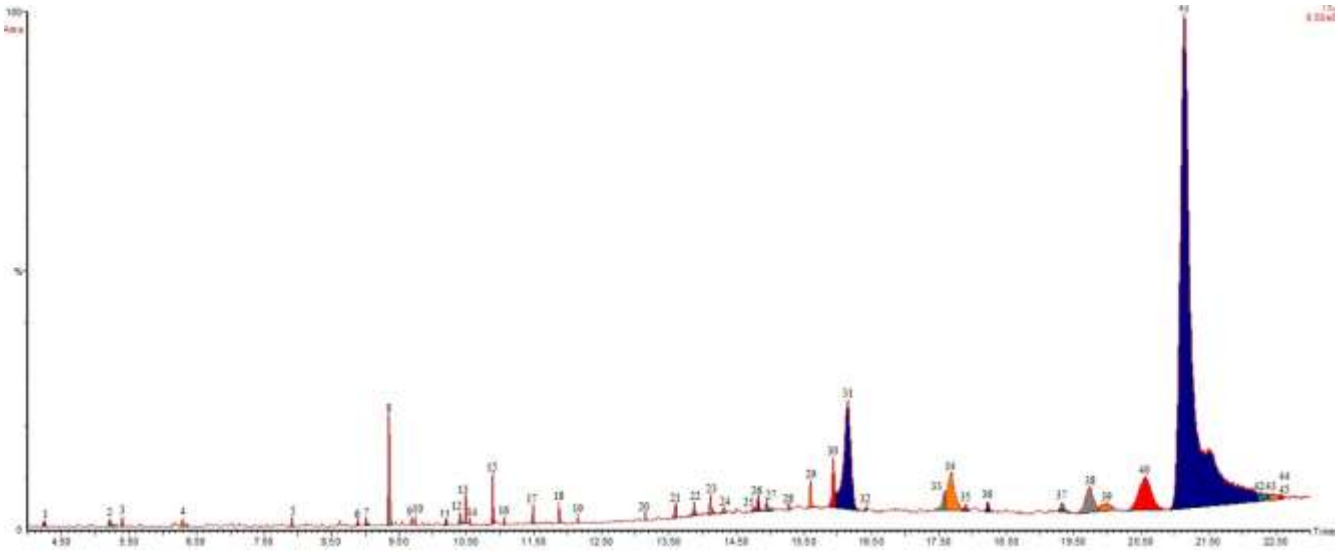

Figure 2. Chromatogram of volatile components in olive oil
Şekil 2. Zeytin yağı uçucu bileşen kromatogramı

This study provides important information regarding the volatile aroma compounds detected in olive oil, particularly concerning the oil's sensory quality and chemical integrity. The identified aldehydes, particularly (E)-2-dekenal, (E)-2-dodesenal, and (E,E)/(E,Z)-2,4-dekadienal, are consistent with lipoxygenase (LOX) pathway products responsible for the characteristic green and fruity aroma of olive oil. The literature reports that these components are commonly found in high-quality natural olive oils and indicate controlled oxidative processes (Angerosa et al., 2004; Kalua et al., 2007).

The unsaturated hydrocarbons identified in the study (e.g., (Z)-3-hexadecen, 8-heptadecen, and 9-octadecen), while having limited direct aromatic effects, contribute to the chemical fingerprint specific to the variety and geographical origin of olive oil (Aparicio and Morales, 1998).

Terpenic and isoprenoid structures, particularly squalene, characterize the distinctive unsaponifiable fraction of olive oil, contributing to oxidative stability and indirectly preserving aroma components. The presence of squalene is considered a characteristic indicator of unrefined olive oils (Smith, 2000; Fernández-Cuesta et al., 2013).

The detected alcohols and esters result from enzymatic reactions during the crushing and kneading stages of olive fruit and are reported to be associated with the oil's fresh, positive sensory characteristics. The presence of these components supports the application of appropriate technological processing conditions (Servili et al., 2004).

The detection of certain sulfur-containing or derived compounds is largely dependent on the derivatization agents used in GC–MS analysis, a widely reported analytical phenomenon in the literature. Therefore, the biological or sensory effects of these compounds should be interpreted with caution (Grosch, 2001).

In general, the identified aroma profile is consistent with the distribution of volatile compounds described in the literature for high-quality olive oils. The findings indicate that the olive oil is sensorially balanced, free from oxidative deterioration, and possesses an authentic structure; they support the reliability of GC–MS-based aroma analysis as a tool for quality and origin assessment (Kalua et al., 2007; Angerosa et al., 2004).

Antioxidant activity results

The radical scavenging results of virgin olive oil are given in Table 8, while the reduction results are given in Table 9.

Tablo 7. The volatile components found in virgin olive oil
Çizelge 7. Sızma zeytin yağında bulunan uçucu bileşenler

Peak	Component Name	Time	Area	Area (%)
1.	3-Hexadecene, (Z)-	4.230	456554	0,1503
2.	Pentadecafluorooctanoic Acid, Octyl Ester	5.210	736571	0,2426
3.	Silane, [Thiobis(Methylene)]Bis[Thiomethyl	5.397	380171	0,1252
4.	6-Octen-1-Ol, 3,7-Dimethyl-, Formate	6.298	617259	0,2033
5.	Oxalic Acid, 2tbdms Derivative	7.918	412699	0,1359
6.	Hydroperoxide, 1-Methylpentyl	8.892	303385	0,0999
7.	2,6-Pyrazinediamine	9.012	351273	0,1157
8.	2-Decenal, (E)-	9.352	5888638	1,9396
9.	Cyclopropanemethanol, 2-Methyl-2-(4-Methyl-3-Pentenyl)-	9.692	425465	0,1401
10.	Mercaptoacetic Acid, 2tms Derivative	9.739	455211	0,1499
11.	8-Heptadecene	10.199	212568	0,0700
12.	2-Dodecenal, (E)-	10.406	558421	0,1839
13.	2,4-Decadienal, (E,Z)-	10.499	2054865	0,6768
14.	9-Octadecyne	10.552	201279	0,0662
15.	2,4-Decadienal, (E,E)-	10.892	1951597	0,6428
16.	Mercaptoacetic Acid, 2tms Derivative	11.066	421288	0,1387
17.	2,2,4-Trimethyl-1,3-Pentanediol Diisobutyrate	11.486	701973	0,2312
18.	(2e,6e,10e)-3,7,11,15-Tetramethylhexadeca-2,6,10,14-Tetraen-1-Yl Format	11.873	810635	0,2670
19.	Boric Acid, 3tms Derivative	12.153	419845	0,1382
20.	Boric Acid, 3tms Derivative	13.153	402565	0,1326
21.	Bicyclo[3.3.1]Nonan-9-One, 1,2,4-Trimethyl-3-Nitro-, (2-Endo,3-Exo,4-Exo)-	13.607	624586	0,2057
22.	Tetradecanoic Acid, 12-Methyl-, Methyl Ester, (S)-	13.880	685963	0,2259
23.	Hexadecanoic Acid, Ethyl Ester	14.120	1256622	0,4139
24.	Methanol, [6,8,9-Trimethyl-4-(1-Propenyl)-3-Oxabicyclo[3.3.1]Non-6-En-1-Y	14.327	607155	0,1999
25.	2-[4-Methyl-6-(2,6,6-Trimethylcyclohex-1-Enyl)Hexa-1,3,5-Trienyl]Cycloh	14.774	611263	0,2013
26.	1,3,3-Trimethyl-2-Hydroxymethyl-3,3-Dimethyl-4-(3-Methylbut-2-Enyl)-C	14.827	1168595	0,3849
27.	1-Methylene-2b-Hydroxymethyl-3,3-Dimethyl-4b-(3-Methylbut-2-Enyl)-C	14.947	857955	0,2826
28.	1,3-Bis-T-Butylperoxy-Phthalan	15.281	432568	0,1424
29.	1-Hexyl-2-Nitrocyclohexane	15.601	1753893	0,5777
30.	Bicyclo[4.1.0]Heptane, 7-Pentyl	15.941	3933532	1,2956
31.	P-Cymene-2,5-Diol	16.161	25337206	8,3458
32.	1-Methylene-2b-Hydroxymethyl-3,3-Dimethyl-4b-(3-Methylbut-2-Enyl)-C	16.428	400125	0,1317
33.	5,9,13-Pentadecatrien-2-One, 6,10,14-Trimethyl-, (E,E)-	17.588	2753893	0,9071
34.	Squalene	17.675	8206448	2,7031
35.	Methanol, [6,8,9-Trimethyl-4-(1-Propenyl)-3-Oxabicyclo[3.3.1]Non-6-En-1-	17.908	414568	0,1365
36.	2,6,10,14-Hexadecatetraen-1-Ol, 3,7,11,15-Tetramethyl-, Acetate, (E,E,E	18.228	495865	0,1633
37.	1-Methylene-2b-Hydroxymethyl-3,3-Dimethyl-4b-(3-Methylbut-2-Enyl)-C	19.329	3258889	1,0734
38.	Eicosanoic Acid	19.742	7568958	2,4931
39.	1,6:3,4-Dianhydro-2-Deoxy-.Beta.-D-Lyxo-Hexopyranose	20.009	3258889	1,0734
40.	1,3-Benzenedicarboxylic Acid, Bis(2-Ethylhexyl) Ester	20.556	13751427	4,5296
41.	Cis-11-Eicosenoic Acid	21.143	197217152	64,9616
42.	1-Hexyl-2-Nitrocyclohexane	22.276	3385468	1,1151
43.	2-Dodecen-1-Yl(-)Succinic Anhydride	22.383	3211225	1,0577
44.	1-Hexyl-2-Nitrocyclohexane	22.443	3014252	0,9928
45.	1-Hexyl-2-Nitrocyclohexane	22.576	2021568	0,6658
		Total	**303590202**	**100**

Tablo8. Radical scavenging results of virgin olive oil (µg/mL)
Çizelge 8. Sızma zeytin yağının radikal giderme sonuçları (µg/mL)

Sample and standards	DPPH· scavenging		ABTS·+ scavenging	
	IC_{50}	r^2	IC_{50}	r^2
BHA	13.29±0.12	0.9917	16.96±0.49	0.9912
BHT	15.81±0.27	0.9895	17.64±0.63	0.9963
α-Tokoferol	8.13±0.10	0.9783	11.21±0.21	0.9756
Trolox	11.21±0.11	0.9961	16.65±0.33	0.9812
Ascorbic Acid	5.93±0.09	0.9889	13.33±0.12	0.9755
Olive oil	10.21±0.12	0.9977	12.11±0.13	0.9921

All values are expressed as mean ± SD ($p < 0.05$ is considered significant) and are the average of three parallel measurements (n = 3).

Tablo 9. Reduction results of virgin olive oil (µg/mL)
Çizelge 9. Sızma zeytinyağının indirgeme sonuçları (µg/mL)

Sample and standards	Fe^{3+} reduction		CUPRAC		FRAP	
	λ_{700}	r^2	λ_{450}	r^2	λ_{593}	r^2
BHA	3.043±0.060	0.9916	2.215±0.063	0.9935	2.217±0.007	0.9974
BHT	2.650±0.089	0.9858	2.017±0.058	0.9856	2.035±0.092	0.9883
α-Tokoferol	2.700±0.156	0.9883	1.606±0.056	0.9791	2.315±0.003	0.9769
Trolox	1.757±0.109	0.9763	1.530±0.062	0.9872	1.580±0.024	0.9790
Ascorbic Acid	2.813±0.032	0.9976	2.530±0.062	0.9763	3.009±0.168	0.9815
Olive oil	1.285±.0.133	0.9868	1.521±0.151	0.9912	1.448±0.008	0.9821

All values are expressed as mean ± SD ($p < 0.05$ is considered significant) and are the average of three parallel measurements (n = 3).

In this study, the antioxidant capacity of olive oil was evaluated using the DPPH· and ABTS·+ radical scavenging methods, and the results were compared with commonly used synthetic and natural antioxidant standards. Olive oil demonstrated strong free radical scavenging potential, with low IC_{50} values in both assays. In the DPPH· assay, the IC_{50} value for olive oil (10.21 µg/mL) was lower than those of synthetic antioxidants such as BHA and BHT; however, it was close to the levels observed for potent natural antioxidants such as α-tocopherol and ascorbic acid. Similar trends are consistent with previous studies reporting that natural olive oils rich in phenolic compounds exhibit strong DPPH· scavenging activity (Visioli & Galli, 1998; Covas et al., 2006).

In the ABTS·+ assay, olive oil exhibited a higher antioxidant capacity (IC_{50} value of 12.11 µg/mL) compared to Trolox, BHA, and BHT. The ABTS·+ method's sensitivity to both hydrophilic and lipophilic antioxidants explains olive oil's strong performance in this test. One of the main reasons for the observed difference is that Trolox is a single-component, water-soluble reference standard, whereas olive oil contains numerous lipophilic and hydrophilic components, including phenolics, tocopherols, and squalene (Re et al., 1999; Kalua et al., 2007).

Hydroxytyrosol and tyrosol derivatives found in olive oil can neutralize free radicals through both hydrogen atom transfer and electron transfer mechanisms. Furthermore, lipophilic antioxidants such as tocopherols and squalenhave been reported to e exhibit synergistic effects with phenolic compounds, thereby increasing total antioxidant capacity (Gulcin, 2020; Boskou, 2015; Johnson and Decker, 2015).

The high determination coefficients ($r^2 \geq 0.97$) obtained in both analyses support the reliability of the experimental results. The consistent results obtained from the DPPH· and ABTS·+ tests demonstrate that olive oil exhibits consistent and balanced antioxidant behavior in different radical systems.

In conclusion, this study demonstrates that olive oil is not only a nutritional lipid source but also d potent natural, multi-component antioxidant. The findings support the biochemical basis for olive oil's protective effects against oxidative stress-related diseases and once again emphasize its importance as a functional food.

In this study, the antioxidant potential of virgin olive oil was comprehensively determined using complementary methods based on free radical scavenging (DPPH· and ABTS·+) and reducing power (Fe^{3+} reduction, CUPRAC, and FRAP).

Fe^{3+} reduction, CUPRAC, and FRAP analyses revealed that the reduction power of virgin olive oil was lower than that of single-component, strong redox-active standards such as ascorbic acid and BHA. This result indicates that the antioxidant activity of olive oil is based on a moderate, stable, and continuous redox capacity rather than rapid,

high electron transfer. In particular, the FRAP method's sensitivity to electron transfer mechanisms in acidic environments only partially reflects the reduction potential of phenolic structures (Benzie & Strain, 1996).

The fact that the CUPRAC test showed that the virgin olive oil exhibited values close to Trolox and α-tocopherol indicates its antioxidant potential. The CUPRAC method stands out as a method that more accurately t reflects the true antioxidant capacity of olive oil due to its wide pH range and sensitivity to lipophilic components (Apak et al., 2004).

The high determination coefficients ($r^2 \geq 0.97$) obtained in all reduction capacity analyses indicate that the experimental data are linear and reliable. The differences observed between radical scavenging tests and reduction capacity tests are not a contradiction; rather, they indicate that olive oil is a natural antioxidant system with multiple mechanisms of action. It is widely reported in the literature that phenol-rich olive oils exhibit a more dominant profile in radical scavenging tests and a more balanced profile in reduction tests (Boskou et al., 2015; Gülcin, 2020).

In conclusion, the findings demonstrate that olive oil possesses both a strong free-radical scavenging capacity and a controlled reducing power that delays lipid oxidation. This multifaceted antioxidant behavior supports the evaluation of olive oil as a functional food and strengthens the biochemical basis for its protective effects against oxidative stress-related diseases.

Total phenolic and total flavonoid content

Table 10 presents the results for the total phenolic and total flavonoid contents of extra-virgin olive oil.

Tablo 10. Reduction results of virgin olive oil (µg/mL)
Çizelge 10. Sızma zeytinyağının toplam fenolik ve flavonoid sonuçları (µg/mL)

Sample	Total phenolics (µg GAE / g)	Total flavonoid contents (µg QE / g)
Olive oil	63.212±3.133	47.133±2.121

The values represent the means ± SD of the three parallel measurements. Gallic acid equivalents (GAE) and quercetin equivalents (QE).

In this study, the total phenolic content of olive oil was determined to be 63,212 ± 3,133 µg GAE/g, and the total flavonoid content was determined to be 47,133 ± 2,121 µg QE/g. These values are consistent with the ranges reported in the literature for natural olive oils and indicate that olive oil has a biologically active phenolic profile (Boskou et al., 2012; Covas et al., 2006).

Phenolic compounds in olive oil are among the key determinants of antioxidant activity and play an active role in neutralizing free radicals, particularly through hydroxytyrosol, tyrosol, and their derivatives (Visioli & Galli, 1998). The relatively high TPC and TFC values determined in this study are directly consistent with the low IC_{50} values exhibited by olive oil in the DPPH• and $ABTS^{\bullet+}$ tests. The literature reports strong positive correlations between total phenolic content and radical scavenging capacity (Kalua et al., 2007).

In contrast, in reduction-based tests (Fe^{3+} reduction, FRAP, and CUPRAC), olive oil exhibits lower but balanced activity compared to synthetic antioxidants; this can be explained by the controlled and consistent redox behavior of phenolic compounds (Apak et al., 2004; Frankel, 2014).

The significant level of total flavonoid content indicates that the antioxidant system of olive oil is not limited to simple phenols alone but that different polyphenolic subgroups contribute together. The metal c-chelating and free-radical-scavenging mechanisms of flavonoids provide a complementary effect, particularly in supporting Fe^{3+} reduction and FRAP results (Benzie & Strain, 1996).

Overall, when evaluated in terms of its phenolic and flavonoid content, strong radical scavenging capacity, and balanced reducing power, the findings of this study clearly demonstrate that olive oil is a multi-component, stable, and biologically effective natural antioxidant system. These results support the evaluation of olive oil as a functional food and reinforce the biochemical basis of its protective potential against oxidative stress-related diseases.

Enzyme inhibition assay

The anticholinesterase property of olive oil has been determined by AChE and BChE enzyme inhibition, while its antidiabetic property has been determined by α-glycosidase enzyme inhibition. The results are presented in Table 11.

Extra virgin olive oil exhibited potent enzyme inhibition with IC_{50} values of 4.74 µg/mL for AChE, 6.13 µg/mL for BChE, and 3.11 µg/mL for α-glucosidase. These values indicate that olive oil exhibits significantly higher inhibitory

activitthanto the reference inhibitors, tacrine (for AChE and BChE) and acarbose (for α-glucosidase).

Tablo 11. Enzyme inhibition results of olive oil (µg/mL)
Çizelge 11. Zeytinyağının enzim inhibisyon sonuçları (µg/mL)

Sample and standard	AChE		BChE		α-Glycosidase	
	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2
Olive oil	4.74	0.9895	6.13	0.9978	3.11	0.9974
Tacrine	2.63	0.9883	3.21	0.9883	-	-
Acarbose	-	-	-	-	3.77	0.9890

Tacrine was a common inhibitor of AChE and BChE enzymes, while acarbose was an inhibitor of α-glycosidase enzymes.

AChE and BChE inhibitors that act on the cholinergic system are among the primary targets for the symptomatic treatment of neurodegenerative diseases, particularly Alzheimer's disease (Ellman et al., 1961; Lane et al., 2006; Karageçili et al., 2023). Olive oil's low IC_{50} values on both cholinesterase enzymes indicate not only selectivity but also the potential for bidirectional cholinergic modulation. This is clinically significant, considering that cholinesterase activity increases in the advanced stages of the disease (Giacobini, 2003; Alkhalifa et al., 2024).

When evaluated in terms of α-glucosidase inhibition, olive oil exhibits a lower IC_{50} value compared to acarbose, indicating that it has a strong antidiabetic potential that could suppress postprandial glucose elevation (García-Gavilán et al., 2023; Tao et al., 2013).

In this study, a clear biochemical correlation was observed between the previously reported total phenolic (63,212 µg GAE/g) and total flavonoid (47,133 µg QE/g) contents and strong enzyme inhibition. In particular, hydroxytyrosol, tyrosol, and flavonoid derivatives are widely reported in the literature to be effective as cholinesterase and α-glucosidase inhibitors (Lu et al., 2024; Bulotta et al., 2014). Furthermore, the high antioxidant capacity plays a supportive role in the inhibitory effect by suppressing enzyme activation caused by oxidative stress.

Overall, olive oil stands out in this study as a natural agent with biofunctional and therapeutic properties for the prevention of both neurodegenerative and metabolic diseases due to its multi-target enzyme inhibition, high antioxidant capacity, and rich bioactive compound content (Jiménez-Sánchez et al., 2022; González-Rodríguez et al., 2023; Sain et al., 2022; Navarro-Hortal et al., 2023).

CONCLUSION

The fatty acid profile of the olive oil analyzed in this study clearly reflects the characteristic composition specific to olive oil. According to gas chromatography results, oleic acid was determined to be the highest in total fatty acids, followed by palmitic acid and linoleic acid. The significantly higher proportion of unsaturated fatty acids in the total fatty acids compared to saturated fatty acids indicates that the samples have a nutritionally valuable lipid profile. Furthermore, the detection of polyunsaturated fatty acids such as α-linolenic acid and γ-linolenic acid is important for understanding their biological functions.

Biochemical analyses have revealed that olive oil exhibits a significant inhibitory effect oe AChE, BChE, and α-glucosidase. It is thought that the inhibition of the α-glucosidase enzyme, in particular, is stronger than that on other enzymes and that this effect may stem from the synergistic effect of the fatty acid composition and phenolic compounds naturally found in olive oil. The inhibition of cholinesterase enzymes points to the neuroprotective potential of olive oil.

Antioxidant capacity has demonstrated that olive oil possesses high free radical scavenging and electron donor capacity. This antioxidant activity has been attributed to unsaturated fatty acids, primarily oleic acid, and phenolic compounds.

The limitations of this study are as follows: The fact that only one sample from one region was studied and that in vivo, preclinical, and clinical studies could not be conducted are areas where this study could be further developed.

Consequently, this study clearly demonstrates that olive oil is not merely a nutritional lipid source; it is a functional food exhibiting multifaceted biological effects including enzyme inhibition and antioxidant activity. The results support the potential use of olive oil in preventing metabolic and neurodegenerative diseases, indicating that advanced in vivo and molecular mechanistic studies are needed to confirm these effects.

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