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Evaluation of Lipid Composition and Nutritional Quality of Olive Oil Varieties Using ESI-MS, GC-FID and Chemometrics Techniques

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Olive oil is a highly demanded product renowned for its unique taste and diverse nutrient content, primarily composed of lipids. This study aimed to comprehensively analyze the lipid profiles of four olive varieties (Arbequina, Arbosana, Koroneiki, and Frantoio) and their corresponding commercial olive oils using advanced analytical techniques, namely electrospray ionization mass spectrometry and gas chromatography with flame ionization detector, combined with principal component analysis. The nutritional quality of the olive oils was evaluated based on parameters such as the ratio of saturated acids to polyunsaturated acids, atherogenicity, thrombogenicity, and andhypocholesterolemic/hypercholesterolemic indexes. The study revealed that the primary fatty acids identified were palmitic, stearic, oleic, and linoleic acids, while triolein, palmitodiolein, and stearodiolein emerged as the most abundant triacylglycerol across all samples. Furthermore, the results demonstrated that the commercial olive oil samples exhibited an exceptional nutritional profile. Overall, this study highlights the significance and efficacy of the analytical and statistical techniques employed to unravel the lipid profiles of olive oils, a product of substantial commercial value and susceptible to adulteration. Additionally, it emphasizes the importance of utilizing nutritional quality indices to assess the health benefits associated with olive oil consumption.

Keywords: olives, fatty acids, triacylglycerol, gas chromatography, mass spectrometry

Introduction

Olive oil, extracted from the fruit of the olive tree (*Olea europaea* L.), is one of the most important vegetable oils consumed worldwide due to its nutritional and sensory properties.[1] Olive oil consists of a complex mixture of fatty acids (FAs), triacylglycerols (TAGs), minor components, and volatile compounds, all of which contribute to its quality, nutritional value, and sensory attributes. Various factors, such as fruit varieties, geographical origin, climatic conditions, ripening stage, and processing technology, can influence the composition of olive oil.[1,2]

Consumption of olive oil has been associated with reduced risks of several types of cancer, as well as positive effects on aging and coronary diseases, primarily due to the presence of phenolic compounds and oleic fatty acid (18:1n-9). Extra virgin olive oils possess a broad spectrum of phenolic compounds, with oleuropein being the predominant compound.[3-5] Research[6] suggests that oleuropein and its hydroxytyrosol metabolite exhibit potential antitumor properties and could potentially be employed in cancer treatments. Additionally, olive oil and its phenolic compounds are recognized for their antioxidant, anti-inflammatory, antimicrobial, and other beneficial properties.[6]

Unsaturated fatty acids, particularly oleic acid, contribute significantly to the health benefits of olive oil, including the reduction of low-density lipoprotein cholesterol (LDL) and the elevation of high-density lipoprotein cholesterol (HDL) levels, thereby reducing the risk of cardiovascular disease.[7-10]

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Indicators such as the atherogenicity index (AI), thrombogenicity index (TI), hypocholesterolemic/hypercholesterolemic fatty acid ratio (HH), saturated fatty acids/polyunsaturated fatty acids ratio (SFA/PUFA), and omega-6 to omega-3 ratio (n-6/n-3) are employed to assess the nutritional quality of olive oil.[11]

High-performance liquid chromatography (HPLC) and gas chromatography coupled to a flame ionization detector (GC-FID) are commonly used techniques for characterizing olive oil samples, with GC requiring additional sample preparation steps such as methylation for FA composition analysis.[12] Recently, electrospray ionization mass spectrometry (ESI-MS) has emerged as a valuable tool for identifying the biological origin and detecting adulteration of vegetable oils, including olive oil, with minimal sample preparation. Chemometric approaches, such as principal component analysis (PCA), can aid in classifying oils from different geographic origins and identifying potential adulterations.[13]

This study aims to evaluate the lipid profile and nutritional quality of four olive oil varieties (Arbequina, Arbosana, Koroneiki, and Frantoio), including commercial samples, utilizing ESI-MS and GC-FID techniques in conjunction with PCA.

Experimental

Samples

Four distinct olive varieties, namely Arbequina (ARQ), Koroneiki (K), Arbosana (ARO), and Frantoio (F), were obtained from Coxilha dos Cunhas (Canguçu, Rio Grande do Sul, Brazil, 8°7'50'' S, 52°17'25'' W). Upon reception, each sample was immediately frozen at −18 ºC, and maintained at that temperature until analysis. Additionally, four commercially available olive oils, specifically Arbequina olive oil (AARQ), Koroneiki olive oil (AK), Arbosana olive oil (AARO), and Frantoio olive oil (AF), were purchased from local markets within the same region to undergo further analysis.

It is worth mentioning that the olives were harvested during the same maturation period, according to the outer color of the fruit. This factor was taken into account, in view of the changes in the chemical composition to which the olives are subjected in the different stages of ripening.

Extraction of olive oil

For the oil extraction process, approximately 700 g of olives were homogenized in a food mixer. The resulting slurry was then subjected to total lipid extraction by Bligh and Dyer.[14] For this, 20 g were homogenized with 80 g of distilled water to correct the humidity. Then, 100 mL of chloroform, HPLC grade (Millipore-Sigma, Darmstadt, Germany) and 200 mL of methanol, HPLC grade (Millipore Sigma, Darmstadt, Germany) were added, with magnetic stirring for 2 min. Subsequently, another 100 mL of chloroform were added and the contents stirred for 30 s and finally, it was added 100 mL of distilled water and stirred for 5 min, maintaining the proportion of solvents chloroform/methanol/water at (2:2:1.8 v/v/v), respectively.

To finalize the lipid extraction process, the content was filtered in a Buchner funnel with a vacuum pump to separate liquid and solid matter. The liquid fraction was taken to the separation funnel and after 24 h the lower phase was collected and dried in a rotary evaporator to obtain the lipids. For each olive variety, the experiments were conducted in triplicate.

FA methylation

The preparation of fatty acid methyl esters (FAMEs) followed the method proposed by Hartman and Lago,[15] with modifications by Maia and Rodriguez-Amaya.[16] About 25.0 mg of lipids were weighed in a test tube and 4.0 mL of 0.5 mol L^{-1} NaOH/MeOH were added. Then, the test tube was heated in a water bath (100 °C) for 5 min, with subsequent cooling in running water. Soon after, 5.0 mL of esterifying solution (NH_4Cl/H_2SO_4/MeOH) was added and the heating and cooling process was repeated. Then, 4.0 mL of saturated NaCl solution and 2.0 mL of *n*-hexane were added and the tubes were shaken vigorously. Finally, the internal standard methyl tricosanoate, (23:0, Sigma-Aldrich, Saint Louis, USA) was added and after phase separation, the upper portion was collected and injected into the GC.

TAG profile by direct infusion ESI(+)-MS

The TAG profile of the olive oil samples was determined using a modified method.[17,18] For sample preparation, 50.0 µL of olive oil were mixed with 950.0 µL of chloroform. From this solution, 5.0 µL were combined with 1.0 mL of methanol/chloroform (9:1, v/v) and 20.0 µL of ammonium formate (0.10 mol L^{-1}, prepared in methanol, Sigma-Aldrich, Saint Louis, USA). The TAG profile analysis was performed using a Xevo TQD™ triple quadrupole mass spectrometer (Waters, Milford, Massachusetts, USA) equipped with a Z spray™ electrospray as ionization the source, operating in positive mode (ESI(+)-MS). The mass spectrometer

was set to acquire spectra in the mass/charge range (*m/z*) of 100-1200, with a capillary voltage of +3.00 kV, a cone voltage of 35.0 V, a cone flow rate of 50.0 L h^{-1}, a sample flow rate of 50.0 µL min^{-1}, a desolvation gas flow rate of 450 L h^{-1}, a source temperature of 130 ºC, and a desolvation gas temperature of 250 ºC. Data processing was performed using MassLynx™ software.

Gas chromatography analysis

The preparation of FAMEs followed the method proposed by Hartman and Lago,[15] with modifications by Maia and Rodriguez-Amaya.[16] The FAMEs were separated using GC-FID (GC-Shimadzu-2010 Plus, São Paulo, Brazil) equipped with a fused silica capillary column CP-7420 (Select FAME, 100 m, 0.25 mm internal diameter, and 0.25 µm of film thickness of cyanopropyl/polysiloxane). The carrier gas used was hydrogen (H_2), at a flow rate of 1.2 mL min^{-1}, and nitrogen (N_2) was used as the makeup gas with a flow rate of 30 mL min^{-1}. The flow rate of H_2 and synthetic air flow in the detector flame was 30 and 300 mL min^{-1}, respectively. A volume of 1.0 µL was injected with a split ratio of 1:40. A temperature program was employed, starting with a temperature at 165 ºC held for 18 min, followed by a ramping period of 20 min with a temperature increase rate of 4 ºC min^{-1}. The temperature of the detector was set at 250 °C, while the injector temperature was maintained at 230 °C. Retention times and peak areas of the analytes were determined by integration using Chromquest 5.0 software. The FAs were identified by comparing their retention times with known composition patterns from the FAME Mix (C4-C24, Sigma-Aldrich, Saint Louis, USA). The analyses were carried out in triplicate.

Nutritional quality indexes of lipids

The nutritional quality of the lipids was assessed based on the analysis of FA composition, and several indexes were used for evaluation: (*i*) atherogenicity index (AI); (*ii*) thrombogenicity index (TI); (*iii*) hypocholesterolemic/hypercholesterolenic FA ratio (HH); (*iv*) polyunsaturated FA/saturated FA (PUFA/SFA); and (*v*) omega-6/omega-3 (n-6/n-3) ratio.

The AI and TI indexes reflect the relationship between the main saturated FAs and the main classes of unsaturated FAs.[19] Equation 1 was used to calculate the AI, while equation 2 was used to calculate the TI.

$$AI = \frac{[(4 \times 14{:}0) + 16{:}0]}{MUFA + n\text{-}6 + n\text{-}3} \quad (1)$$

$$TI = \frac{(14{:}0 + 16{:}0 + 18{:}0)}{\left[(0.5 \times MUFA) + (0.5 \times n\text{-}6) + (3 \times n\text{-}3) + \left(\frac{n\text{-}3}{n\text{-}6}\right)\right]} \quad (2)$$

where MUFA is the monounsaturated fatty acid. The HH which is associated with cholesterol metabolism, was calculated using equation 3.[20]

$$HH = \frac{[(18{:}1n\text{-}9 + 18{:}2n\text{-}6) + 18{:}3n\text{-}3 + 20{:}5n\text{-}3 + 22{:}5n\text{-}3 + 22{:}6n\text{-}3]}{(14{:}0 + 16{:}0)} \quad (3)$$

Statistical analysis

The FA composition results obtained by GC-FID are presented as mean ± standard deviation (SD) and were subjected to one-way analysis of variance (ANOVA) tests. Means were compared using Tukey's test, with a significance level of 5% ($p < 0.05$). The data were processed using the GraphPad Prism® software (version 5.0).[21] Additionally, PCA was performed on the FA composition results obtained by GC-FID using R software (version 4.2.3).[22]

Results and Discussion

TAG profile obtained by ESI(+)-MS

The direct infusion ESI(+)-MS technique is a highly effective analytical method widely employed for the characterization of various products, including coconut oil, olive oil, soybean, corn, canola, cottonseed oils, sunflower oil, avocado, omega-3 supplements, beer, whisky, cachaça, cosmetics, biodiesel, human milk,[23-33] and others. It has demonstrated its versatility in analyzing complex mixtures, offering valuable information about their composition and structure.

In this study, the direct infusion ESI(+)-MS analysis proved to be a successful method for identifying the predominant TAGs present in commercial olive oil varieties, namely Arbosana, Arbequina, Koroneiki, and Frantoio. The TAG profile obtained through this analysis serves as an efficient method for classifying and characterizing monovarietal oils, highlighting its importance in understanding the composition of these oils.[34] Figure 1 illustrates the TAG profile of olive oil extracted from the four different olive varieties (Arbequina, Koroneiki, Arbosana, and Frantoio), further emphasizing the significance of the TAG profile in understanding the composition of these oils.

Additionally, Figure 2 presents the commercial olive oil samples corresponding to their respective varieties.

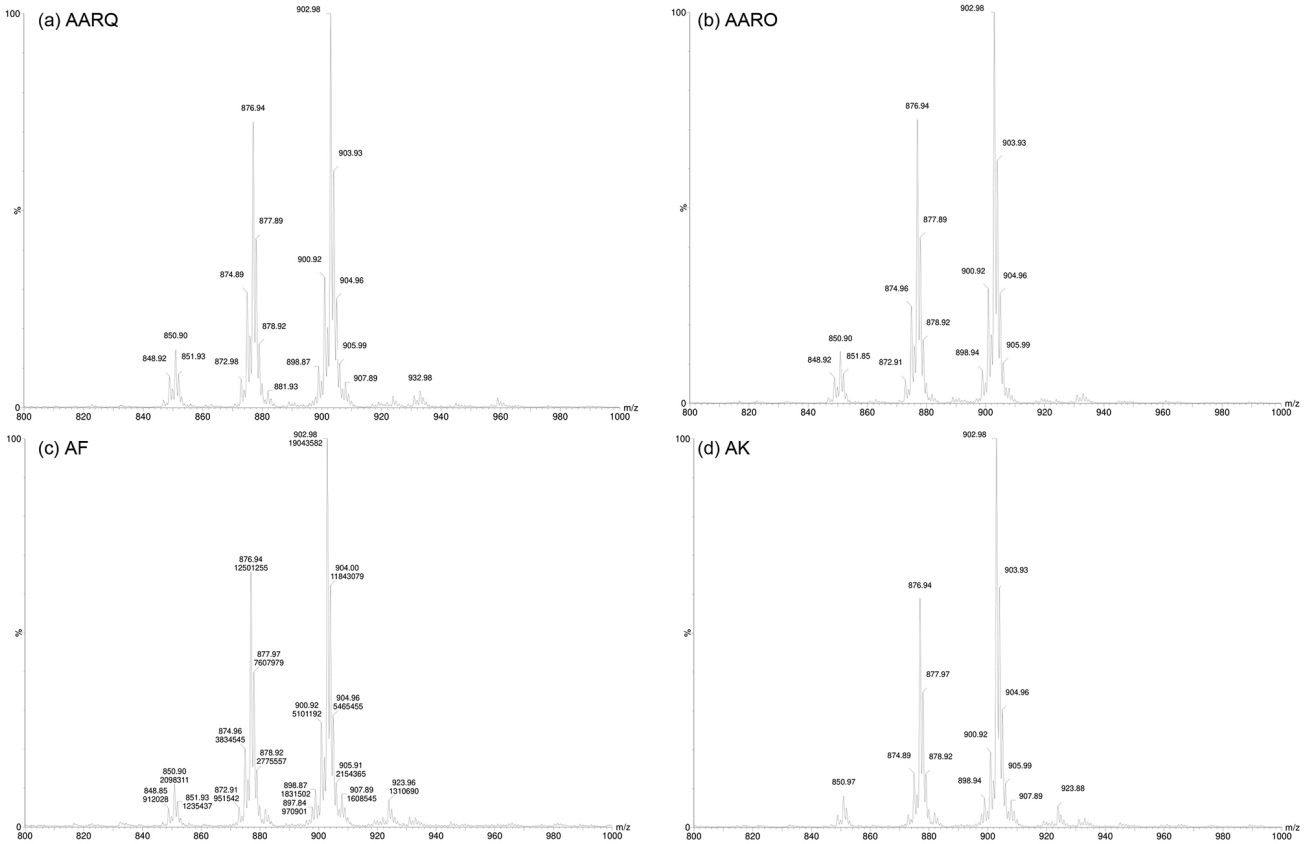

Figure 1. TAG profile by direct infusion ESI(+)-MS of the (a) Arbequina (AARQ); (b) Arbosana (AARO); (c) Frantoio (AF); (d) Koroneiki (AK).

Table 1 provides a comprehensive list of the major TAGs identified in the analyzed samples using both the direct infusion ESI(+)-MS and LAMES platform.[35]

The identification of TAGs was performed irrespective of the fatty acid positions within the TAG molecule. The abbreviations P, O, L, S, Ln, and A correspond to palmitic acid, oleic acid, palmitoleic acid, linoleic acid, stearic acid, linolenic acid, and arachidic acid, respectively.

The major TAGs identified in the analyzed samples include triolein (OOO, 902 *m/z*), palmitodiolein (OOP, 876 *m/z*), and stearodiolein (SOO, 904 *m/z*), which are consistent with previous studies.[36,37] Additionally, other important TAGs were also detected, such as dioleolinolein (OOL, 900 *m/z*), palmitooleolinolein (PLO, 874 *m/z*), dipalmitoolein (POP, 850 *m/z*), trilinolein (LLL, 896 *m/z*), oleolinoleolinolenin (OLLn, 896 *m/z*), oleodilinolein (OLL, 898 *m/z*), dioleolinolenin (OOLn, 898 *m/z*), palmitodilinolein (PLL, 872 *m/z*), palmitooleolinolenin (POLn, 872 *m/z*), dipalmitolinolein (PPL, 848 *m/z*), and palmitostearoolein (POS, 878 *m/z*).

It is noteworthy that several studies have shown that the TAG content of olive oils is influenced by several factors, including the type of cultivar, stage of fruit maturation, climatic conditions and geographic region. For example, research conducted on Turkish monovarietal olive oils revealed variations in TAG composition attributed to different cultivars and geographic regions. Similarly, a study on French virgin olive oil reported significant differences in TAG content among cultivars, with percentages ranging from 27.32 to 58.76% for OOO, 14.69 to 27.65% for POO, 7.48 to 23.27% for LOO, and 2.16 to 11.71% for PLO.[38] These findings highlight the impact of various factors on the TAG composition of olive oils, emphasizing the need for comprehensive analysis and characterization.

Analysis of fatty acid composition

The FA composition of olive oil varieties (AK, AF, AARO, and AARQ) and their corresponding commercial samples (K, F, ARO, and ARQ) was determined by GC-FID. The results obtained for the FA composition are presented in Table 2.

The SFA, MUFA, and PUFA contents ranged from 16.79 to 18.78%, 72.42 to 77.76%, and 4.79 to 10.01% among the samples, respectively. Significant differences ($p < 0.05$) were observed in the FA composition between the commercial olive oil samples and their corresponding olive oil varieties. Notably, ABQ and AABQ, ABO and AABO, as well as F and AF, demonstrated significant differences in terms of SFA, MUFA, and PUFA content. Although

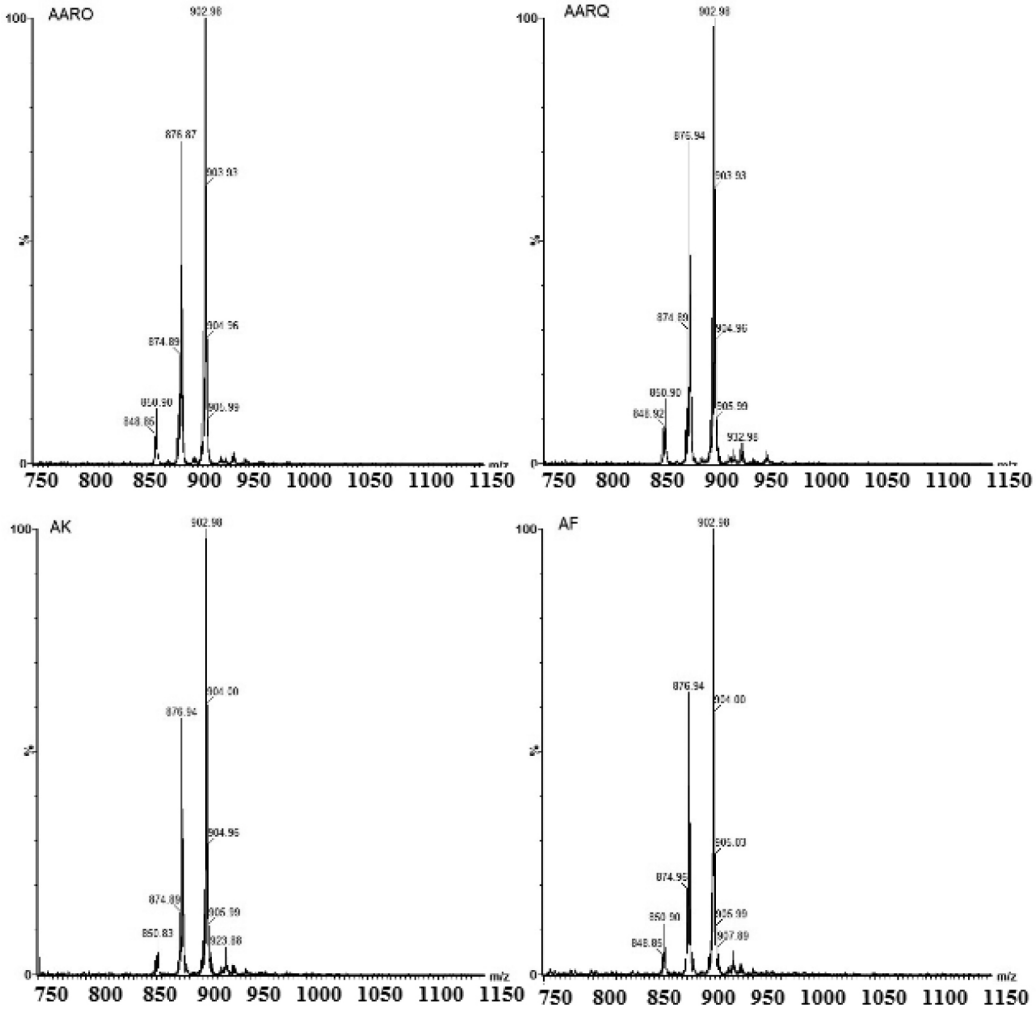

Figure 2. TAG profile of commercial olive oils from Arbosana (AARO), Arbequina (AARQ), Koroneiki (AK), and Frantoio (AF) varieties analyzed by direct infusion ESI(+)-MS.

Table 1. Major TAGs identified in the olive oil samples by direct infusion ESI(+)-MS and LAMES platform

$[TAG + NH_4]^+$	TAG[a]	Molecular formula
848	PPL	$C_{53}H_{98}O_6$
850	POP	$C_{53}H_{100}O_6$
872	PLL / POLn	$C_{55}H_{98}O_6$
874	PLO	$C_{55}H_{100}O_6$
876	OOP	$C_{55}H_{102}O_6$
878	POS	$C_{55}H_{104}O_6$
896	LLL / OLLn	$C_{57}H_{98}O_6$
898	OLL / OOLn	$C_{57}H_{100}O_6$
900	OOL / LLS	$C_{57}H_{102}O_6$
902	OOO	$C_{57}H_{104}O_6$
904	SOO	$C_{57}H_{106}O_6$
906	SSO	$C_{57}H_{108}O_6$
930	AOL	$C_{59}H_{108}O_6$
932	OOA	$C_{59}H_{110}O_6$
934	SOA	$C_{59}H_{112}O_6$

[a]TAGs represent specific triacylglycerol compounds identified in the samples. P: palmitic acid; O: oleic acid; L: linoleic acid; S: stearic acid; Ln: linolenic acid; A: arachidic acid.

the SFA content of K and AK did not differ significantly, substantial differences were found in the MUFA and PUFA content between these samples. This indicates that the olive oil extraction process may influence the FA composition of the olive oils. Factors such as temperature, time, and equipment used during extraction can impact the MUFA and PUFA content. Moreover, it is important to acknowledge that the FA composition of olive oil can vary based on variables such as olive variety, harvesting time, and processing methods.

Analyzing the individual FA, it can be observed that the most abundant FA in all samples was oleic acid, ranging from 70.56 (AARQ) to 76.25% (AK). Other major fatty acids included palmitic acid (P, 16:0), linoleic acid (L, 18:2n-6), and stearic acid (S, 18:0). The palmitoleic acid (Po, 16:1n-7), linolenic acid (Ln, 18:3n-3), arachidic acid (A, 20:0) and gadoleic acid (G, 20:1n-11) are present in smaller quantities, representing less than 4% in all samples. Margaric acid (M, 17:0) and lignoceric acid

Table 2. Fatty acid composition of the commercial olive oil samples and the olive oil varieties determined by GC-FID

Fatty acid composition / %	Sample							
	Olive oil				Commercial olive oil			
	ARQ	ARO	K	F	AARQ	AARO	AK	AF
16:0	16.40 ± 0.26^{aA}	16.10 ± 0.10^{aB}	14.57 ± 0.01^{bC}	15.02 ± 0.01^{aD}	16.17 ± 0.01^{aA}	15.70 ± 0.02^{bB}	14.75 ± 0.01^{aCDE}	14.68 ± 0.03^{bCE}
16:1n-7	1.44 ± 0.01^{aG}	1.62 ± 0.01^{aF}	1.20 ± 0.10^{aA}	1.25 ± 0.01^{aAB}	1.79 ± 0.01^{bE}	1.31 ± 0.00^{bBC}	1.19 ± 0.00^{aABD}	1.25 ± 0.03^{aABCD}
17:0	0.24 ± 0.01^{aA}	0.27 ± 0.01^{aB}	0.21 ± 0.01^{aC}	0.22 ± 0.01^{aAC}	0.26 ± 0.01^{aBD}	0.28 ± 0.01^{aBD}	ND^{bE}	ND^{bE}
18:0	1.60 ± 0.10^{aA}	1.82 ± 0.01^{aB}	2.14 ± 0.06^{bF}	1.54 ± 0.06^{bAC}	1.70 ± 0.01^{aABD}	1.74 ± 0.01^{bBD}	2.30 ± 0.01^{aE}	1.66 ± 0.01^{aACD}
18:1n-9	71.20 ± 0.10^{aA}	70.75 ± 0.01^{bB}	73.24 ± 0.06^{bC}	71.18 ± 0.01^{aA}	70.56 ± 0.06^{bD}	72.81 ± 0.00^{aE}	76.25 ± 0.00^{aF}	75.65 ± 0.01^{aG}
18:2n-6	6.53 ± 0.21^{bA}	7.13 ± 0.06^{aB}	6.47 ± 0.15^{aA}	9.02 ± 0.01^{aC}	8.22 ± 0.05^{aD}	6.54 ± 0.01^{bA}	3.73 ± 0.01^{bE}	3.98 ± 0.00^{bF}
18:3n-3	1.72 ± 0.01^{aB}	1.34 ± 0.01^{aA}	1.32 ± 0.01^{aA}	0.98 ± 0.01^{bC}	0.72 ± 0.01^{bD}	0.84 ± 0.00^{bE}	1.06 ± 0.00^{bF}	1.90 ± 0.07^{aG}
20:0	0.50 ± 0.10^{aA}	0.34 ± 0.02^{aB}	0.33 ± 0.02^{bB}	0.30 ± 0.01^{bBC}	0.26 ± 0.05^{bBC}	0.36 ± 0.00^{aBC}	0.40 ± 0.01^{aABC}	0.44 ± 0.01^{aAB}
20:1n-11	0.33 ± 0.02^{aA}	0.25 ± 0.01^{bB}	0.31 ± 0.01^{aA}	0.31 ± 0.01^{bA}	0.31 ± 0.02^{aA}	0.41 ± 0.00^{aC}	0.31 ± 0.01^{aA}	0.44 ± 0.01^{aD}
24:0	0.19 ± 0.01^{aA}	0.18 ± 0.01^{aA}	0.22 ± 0.01^{aB}	0.11 ± 0.01^{aC}	ND^{bD}	ND^{bD}	ND^{bD}	ND^{bD}
SFA	18.63 ± 0.02^{aB}	18.78 ± 0.07^{aC}	17.46 ± 0.01^{aA}	17.25 ± 0.01^{aD}	18.39 ± 0.03^{bE}	18.09 ± 0.01^{bF}	17.45 ± 0.02^{aA}	16.79 ± 0.04^{bG}
MUFA	73.06 ± 0.01^{aC}	72.61 ± 0.02^{bA}	74.64 ± 0.06^{bB}	72.42 ± 0.29^{bA}	72.66 ± 0.05^{bA}	74.53 ± 0.00^{aB}	77.76 ± 0.01^{aD}	77.34 ± 0.04^{aE}
PUFA	8.31 ± 0.01^{aA}	8.53 ± 0.01^{aB}	7.92 ± 0.01^{aC}	10.01 ± 0.01^{aD}	8.95 ± 0.04^{bE}	7.38 ± 0.01^{bF}	4.79 ± 0.02^{bG}	5.88 ± 0.08^{bH}

Results were expressed as means ± standard deviation (SD) of three replicates. Values with different uppercase letters in the same row are significantly different ($p < 0.05$) by Tukey's test. Values with different lowercase letters in the same row indicate the statistically significant differences ($p < 0.05$) between the mean values of different fatty acids between the commercial olive oil samples and the corresponding olive oil varieties by the Tukey's test. ND: not detected; K: Koroneiki; F: Frantoio; ARO: Arbosana; ARQ: Arbequina; AK: Koroneiki olive oil; AF: Frantoio olive oil; AARO: Arbosana olive oil; AARQ: Arbequina olive oil; SFA: saturated fatty acid; MUFA: monounsatured fatty acid; PUFA: polyunsaturated fatty acid.

(Lg, 24:0) appear in amounts below 0.3% in all the analyzed olive oils.

Other studies[38,39] have reported similar findings, for example, the percentage of oleic acid was 76.70% (Koroneiki), 74.39% (Arbosana), and 67.70% (Arbequina). The percentage of palmitic acid was 16.69% (Arbequina), 14.28% (Arbosana), and 13.89% (Koroneiki), while the percentage of linoleic acid was 10.40% (Arbequina), 6.03% (Arbosana), and 5.76% (Koroneiki). In another study[39,40] investigating the FA composition and oxidative stability of olive oils extracted from olive trees in Southern Brazil, Arbequina exhibited the major FAs as oleic acid (59.15%), followed by palmitic acid (18.51%), and linoleic acid (15.79%), while Frantoio had percentages of oleic acid (66.11%), palmitic acid (15.12%), and linoleic acid (13.94%). For Koroneik, the values were 76.47, 12.82, and 5.52% for oleic acid, palmitic acid, and linoleic acid, respectively.[38-40]

Furthermore, an evaluation of the FA composition of Arbequina in different crop years revealed that oleic acid was the predominant FA, ranging from 77.66 to 82.93%. These values were higher than those obtained in the present study (70.56 to 71.20%). It should be noted that the amount of oleic acid can be influenced by temperature during olive oil extraction. Lower extraction temperatures can lead to an increase in oleic acid content, and even a 1 °C increase in temperature can result in reductions of up to 2% in the content of this compound.[41]

Overall, the results highlight the variations in the fatty acid composition of olive oil varieties and their commercial counterparts. These differences may be attributed to various factors such as olive cultivars, processing methods, and storage conditions. Further studies are needed to investigate the implications of these compositional variations on the sensory attributes, shelf life, and nutritional properties of olive oils.[38-41]

Principal component analysis (PCA)

PCA was performed using the data obtained from GC-FID to explore the relationship between commercial olive oil samples and the oil extracted from their respective olives. The results of the PCA are illustrated in Figure 3.

The PCA yielded an explanation of approximately 96% of the total variance, with 72.92% attributed to principal component 1 (PC1) and 23.39% to principal component 2 (PC2).

Based on the PCA results, it is evident that certain samples displayed positive or negative correlations with specific principal components (PC1 and PC2). Samples AF and K exhibited positive correlations with both PC1 and PC2, indicating that their fatty acid compositions differed from the other oils. Sample AK showed a positive correlation with PC1 but a negative correlation with PC2,

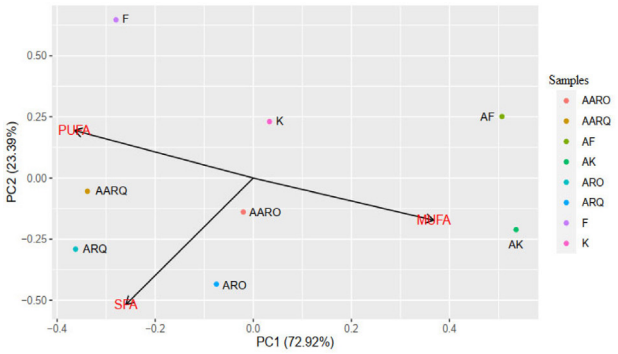

Figure 3. Principal component analysis (PCA) of commercial olive oil samples and their corresponding olive oils. K: Koroneiki; F: Frantoio; ARO: Arbosana; ARQ: Arbequina; AK: Koroneiki olive oil; AF: Frantoio olive oil; AARO: Arbosana olive oil; AARQ: arbequina olive oil; SFA: saturated fatty acid; MUFA: monounsatured fatty acid; PUFA: polyunsaturated fatty acid.

suggesting a distinct fatty acid profile compared to the other samples. Samples ARQ, AARQ, ARO, and AARO clustered together and displayed negative correlations with both PC1 and PC2, indicating a higher similarity in terms of fatty acid composition among these samples.

On the other hand, samples K and F exhibited highly distinct profiles compared to the other oils as they did not group with any other sample. This indicates the possibility of having unique fatty acid profiles that differentiate them from the other oils.

Additionally, the direction of the vectors in the PCA plot provided insights into the fatty acid composition of the samples. ARO and ARQ samples exhibited a higher concentration of SFA, whereas AK and AF samples had a higher concentration of MUFA. Furthermore, samples ARQ, AARQ, and F showed a higher content of PUFA. This information holds practical value for quality control and product development within the olive oil industry. Understanding the fatty acid profiles of different oils and their relationship to sensory and nutritional properties can aid in the identification and characterization of oils, enabling informed decision-making and promoting product optimization.

Nutritional properties

The nutritional quality of olive varieties and commercial olive oil samples were evaluated using various nutritional indexes, including PUFA/SFA ratio, n-6/n-3 ratio, AI, TI, and HH.

Table 3 presents the results of these indexes, which provide insights into the nutritional quality of the foods based on their fatty acid compositions.

The AI and TI are indexes that assess the potential impact of a food's fatty acid composition on cardiovascular health. Lower values of AI and TI are considered desirable as they indicate a lower risk of developing cardiovascular diseases.[42-44] The AI values ranged from 0.18 to 0.20, while the TI values ranged from 0.06 to 0.08. These results suggest that all the analyzed olive oil samples have a favorable impact on cardiovascular health.

Table 3. Nutritional quality of olive oils varieties and commercial olive oils

Sample	PUFA/SFA	n-6/n-3	AI	TI	HH
ARQ	0.45	3.80	0.20	0.07	4.84
ARO	0.44	5.32	0.20	0.08	4.92
K	0.48	4.90	0.18	0.07	5.56
F	0.48	9.24	0.18	0.06	5.40
AARQ	0.45	11.41	0.20	0.08	4.92
AARO	0.46	7.76	0.19	0.07	5.11
AK	0.48	3.51	0.18	0.08	5.49
AF	0.50	2.10	0.18	0.06	5.55

AI: atherogenicity index; TI: thrombogenicity index; HH: hypocholesterolemic/hypercholesterolenic fatty acid ratio; PUFA/SFA: polyunsaturated fatty acid/saturated fatty acid; n-6/n-3: omega-6/omega-3; K: Koroneiki; F: Frantoio; ARO: Arbosana; ARQ: Arbequina; AK: Koroneiki olive oil; AF: Frantoio olive oil; AARO: Arbosana olive oil; AARQ: Arbequina olive oil.

The HH values in this study ranged from 4.84 to 5.56, indicating that all the olive oil samples have the potential to contribute to the maintenance of healthy cholesterol levels.

The PUFA/SFA ratio, ranging from 0.44 to 0.50, indicates a favorable nutritional quality of the olive oil varieties and commercial olive oils analyzed in this study. Higher values of the PUFA/SFA ratio are typically associated with improved health outcomes due to the beneficial effects of PUFA on the body. The relatively high PUFA/SFA ratios observed in all the samples suggest that these olive oil varieties and commercial olive oils are abundant in polyunsaturated fatty acids, which have the potential to lower blood cholesterol levels and contribute to the prevention of cardiovascular diseases.[45]

The n-6/n-3 ratio, which ranged from 2.10 to 11.41 in the analyzed samples, provides insights into the balance between omega-6 and omega-3 fatty acids. A lower n-6/n-3 ratio is generally considered more desirable as it indicates a higher intake of omega-3 fatty acids relative to omega-6 fatty acids. This balance is associated with various health benefits, as it can reduce the incidence of chronic diseases involving inflammatory processes, such as cardiovascular diseases, cancer, and rheumatoid arthritis.[46] In this study, sample AF exhibited the lowest n-6/n-3 ratio (2.10), suggesting a relatively favorable balance between these two types of fatty acids. This indicates that consumption of this olive oil may contribute to a healthier dietary profile. On the other hand, the

commercial sample AARQ had the highest n-6/n-3 ratio (11.41), indicating a higher proportion of omega-6 fatty acids relative to omega-3 fatty acids. This suggests that regular consumption of this particular olive oil may have a less favorable impact on human health.

Overall, the results demonstrate that the analyzed olive oil and commercial olive oils possess favorable nutritional profiles. These oils are characterized by high PUFA/SFA ratios, balanced n-6/n-3 ratios, low AI and TI values, and significant hypocholesterolemic potential (as indicated by the HH index). These findings emphasize the potential health benefits associated with the consumption of these olive oil varieties and commercial olive oils, supporting their use in a balanced and nutritious diet.

Conclusions

This study successfully applied ESI-MS and GC-FID techniques for the analysis of lipids in olive oil, providing valuable insights into the FA composition and nutritional quality of the studied samples. The major TAGs identified in all samples, triolein (OOO, *m/z* 902), palmitodiolein (OOP, *m/z* 876), and stearodiolein (SOO, *m/z* 904), can serve as potential markers for olive oil quality assessment. The GC-FID analysis and PCA revealed significant differences between the commercial olive oil samples and their corresponding olives in terms of FA composition. Further investigations are warranted to determine the underlying causes of these differences and ensure the authenticity of the commercial olive oils. The nutritional quality indexes used in this study indicated that all the studied olive oils possess favorable nutritional profiles, which could have a positive impact on consumer's overall health. Notably, the olive oil varieties ARQ, AK, and AF exhibited lower n-6/n-3 ratios and higher HH values, suggesting their potential health benefits. Overall, this research underscores the importance of understanding the nutritional and lipid quality of olive oil, considering its recognized health benefits. The employed techniques, including ESI-MS, GC-FID, and PCA, proved to be effective in evaluating the studied olive oils, and they can be utilized in the assessment of other vegetable oils as well. Further studies in this field will contribute to enhancing our knowledge and promoting the use of high-quality and nutritionally valuable oils in the food industry and for the well-being of consumers.

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Author Contributions

Alisson de L. Figueiredo was responsible for the software, validation, visualization, writing of the original draft, writing-review and editing; Michele C. Silva for conceptualization, data curation, project administration, and writing the original draft; Patrícia D. S. Santos and Mariana Leôncio in charge of data curation, project administration, software, validation and visualization; Jessica S. Pizzo and Luciana P. Manin for software, visualization, original draft writing, writing-review and editing; Oscar O. Santos and Jesuí V. Visentainer for investigation, project administration, resources, validation, writing original draft and writing-review and editing.

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