

Article

Detection of Olive Oil Adulteration with Corn Oil Based on the Phenolic Compounds Profile Obtained by UHPLC-MS/MS

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Abstract

Unrefined vegetable oils are an important source of bioactive compounds with beneficial effects on health. Therefore, confirming the biological identity of oils is important for ethical and economic reasons. In this study, a method was developed for discriminating vegetable oils based on the phenolic compounds profile obtained by ultrahigh-performance liquid chromatography-mass spectrometry (UHPLC-MS/MS). It was found that extra virgin olive oil has a cinnamic acid content of 2.2 mg/kg (mean value), a much higher value compared to other oils (not detected—0.4 mg/kg), thus being a representative phenolic marker for this oil. From the phenolic compounds profile of different vegetable oils, it can be stated that extra virgin olive oil has a specific phenolic content. However, walnut, sunflower, and corn oils have some similarities regarding the phenolic compounds content (for instance, ellagic acid) and, therefore, these oils can be used as adulterants of extra virgin olive oil. Data analysis, including principal component analysis, hierarchical cluster analysis, and partial least-squares discriminant analysis, demonstrated the discrimination of olive oils from other vegetable oils. Data analysis also allowed the discrimination and classification of olive oil samples adulterated with corn oil when the percentage of adulterant was 1%, with an accuracy of more than 90%.

Keywords: vegetable oil; UHPLC-MS/MS; phenolic compounds; data analysis



Received: 8 October 2025

Revised: 16 November 2025

Accepted: 26 November 2025

Published: 27 November 2025

Citation: Geana, E.-I.; Apetrei, I.M.; Apetrei, C. Detection of Olive Oil Adulteration with Corn Oil Based on the Phenolic Compounds Profile Obtained by UHPLC-MS/MS. *Chemosensors* **2025**, *13*, 408. <https://doi.org/10.3390/chemosensors13120408>

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1. Introduction

Unrefined vegetable oils are an important source of compounds that are important in nutrition and health [1,2]. Among these natural products, virgin olive oil is the main component of the Mediterranean diet and from the chemical point of view it contains a saponifiable fraction (around 98%) and an unsaponifiable fraction (around 2%) [3]. In the saponifiable fraction are included triglycerides of different fatty acids, mainly oleic acid, which is present in the highest percentage (55–83%). The unsaponifiable fraction includes different classes of compounds such as sterols, triterpene compounds, alcohols, tocopherols, and phenolic compounds [4–6].

Among them, phenolic compounds are secondary metabolites that are commonly found in many plants and are currently receiving considerable attention due to their antioxidant properties, which are closely related to the prevention of diseases such as cancer, inflammatory conditions, and cardiovascular diseases [7–9]. Furthermore, these

compounds contribute to extending the shelf life of olive oil, preventing oxidation reactions, thus contributing to the satisfactory organoleptic characteristics of olive oil, including aroma [10–12]. In addition, the quantity and quality of phenolic compounds in olive oil depends on olive variety, degree of ripeness, geographical position, environmental conditions, and also on the extraction technique [13,14].

The extraction processes of extra virgin olive oils are carried out under mild conditions so that these compounds are found in high concentrations (over 250 mg/kg), so that their consumption protects the body against the oxidation of blood lipids, which is one of the main mechanisms for the development of cardiovascular diseases [15].

The methods used for extracting phenolic compounds from olive oil and by-products are solvent extraction, hydrothermal extraction, high-pressure and high-temperature steam reactor, subcritical water extraction, and microwave- and ultrasound-assisted extraction. Industrial interest was focused on the development of processes based on selective, environmentally friendly, and cost-effective extraction techniques [16]. The polar fraction is usually obtained by liquid–liquid extraction and also by solid-phase extraction prior to introduction in the chromatographic column for the separation and identification of phenolic compounds. The solvent usually used in the extraction of phenolic compounds from olive oils is methanol: water in different ratios [17,18].

The separation and determination of individual phenolic compounds in extracts obtained by liquid–liquid extraction (LLE) or solid-phase extraction (SPE) is performed by chromatographic methods, in particular high-performance liquid chromatography (HPLC), coupled especially with diode array detector (DAD) detection, but also electrochemical detection [19–21]. Gas chromatography (GC) analysis is less common due to the need for sample derivatization before instrumental detection [22]. Determination of these compounds by high-resolution mass spectrometry (HRMS) offers improved resolution and stability for precise mass measurements, along with accurate quantification of the compounds [23,24].

Most edible vegetable oils are composed primarily of varying proportions of the same fatty acid or structurally similar fatty acids [25]. Extra virgin olive oil (EVOO) can be significantly adulterated so that it does not meet the requirements for extra virgin quality but still appears to be of extra virgin quality to the consumer [26,27]. The addition of other edible oils to an extra virgin olive oil in moderate or small quantities may be difficult to detect by examining physical or organoleptic characteristics [28–30].

Olive oil is one of the most adulterated food products in the world due to its relatively low production and higher prices compared to vegetable oils obtained from seeds. Due to the high price of EVOO, there is a great temptation to adulterate it in order to increase income. The main types of adulteration of olive oil refer to mislabeling, false declaration of geographical origin, substitution with other types of oils, dilution, and intentional distribution of contaminated products/counterfeiting [30–32]. Vegetable oils with similar chemical composition or with a low price have been mainly used as adulterants of olive oil, for instance, high oleic sunflower, peanut, or avocado oil [33]. Gas chromatography-mass spectrometry (GC/MS) [34], liquid chromatography with diode array detector (HPLC/DAD) or mass spectrometry (HPLC/MS) [35,36], spectroscopic [37,38], near-infrared hyperspectral imaging [39], and sensor or biosensor arrays [40–43] have been developed and implemented to detect olive oil adulteration. To analyze data obtained from instrumental analyses, multivariate data analysis methods are used for discrimination, classification, or establishing correlations [44–48].

In this study, different types of vegetable oils were characterized in terms of phenolic compounds profile by ultrahigh-performance liquid chromatography-mass spectrometry (UHPLC-MS/MS). The identified compounds were quantified and the data were evalu-

ated using multivariate statistical analysis (principal component analysis—PCA, cluster analysis—HCA, and partial least-squares discriminant analysis—PLS-DA) to discriminate vegetable oils according to their botanical origin and to classify virgin olive oils adulterated with corn oil.

2. Materials and Methods

2.1. Solvents and Analytical Standards

The solvents (methanol, water, formic acid, etc.) were purchased from Merck Co. (Darmstadt, Germany) and were of HPLC grade. The phenolic compound standards (apigenin, chrysin, galangin, kaempferol, pinocembrin, isorhamnetin, abscisic acid, gallic acid, syringic acid, p-coumaric acid, vanillic acid, caffeic acid, ferulic acid, 3,4-dihydroxybenzoic acid, chlorogenic acid, ellagic acid, p-hydroxybenzoic acid, t-cinnamic acid, (-)-epicatechin, and (+)-catechin) were purchased from Sigma-Aldrich (St. Louis, MO, USA), Merck Co. (Darmstadt, Germany) or HWI (Ruelzheim, Germany).

2.2. Oil Samples

The vegetable oils studied in this work are the following: extra virgin olive oil (EVOO)—4 samples from different countries of origin (Italy, Spain, Greece, and Bulgaria), walnut oil (W)—2 samples, grape seed oil (GS), pumpkin seed oil (PU), flaxseed oil (F)—2 samples, soybean oil (SO), sesame oil (SE), hemp oil (H), poppy seed oil (PO), sunflower oil (SF)—4 samples, and corn oil (C). All the samples were unrefined; therefore, the polar fraction is representative of the biological source of oil. Also, a controlled falsification experiment of extra virgin olive oil with different percentages of corn oil (from 0.5 to 100%) was carried out according to Table 1.

Table 1. Adulteration of extra virgin olive oil with corn oil.

#	C (g)	EVOO (g)	c%
EVOO	0	20	0
A2	0.1	19.9	0.5
A3	0.2	19.8	1
A4	0.5	19.5	2.5
A5	0.6	19.4	3
A6	1	19	5
A7	1.5	18.5	7.5
A8	2	18	10
A9	3.9	16	19.5
A10	10	10	50
C	20	0	100

C = corn oil; EVOO = extra virgin olive oil; c% = percentage adulterant concentration.

2.3. Extraction of Polar Fraction of Oil Samples

The extraction of the polar fraction from the analyzed vegetable oils was carried out according to the International Olive Council protocol (COI/T.20/DocNo 29, November 2009). For this, 2 g of oil is introduced into an extraction tube and 1 mL of internal standard is added (syringic acid 0.015 mg/mL prepared in a methanol/water mixture 80/20 (v/v)) and then homogenized with a vortex for 30 s. In total, 5 mL of extraction solution (methanol/water 80/20 (v/v)) is added and shaken again in a vortex for 1 min, after which the resulting mixture is subjected to ultrasonic extraction in an ultrasonic bath for 15 min at room temperature. Subsequently, the sample is centrifuged at 5000 rpm for 25 min. An aliquot of the supernatant phase is filtered through a 1 mL plastic syringe using nylon syringe filters (0.45 µm) before injection into the HPLC system. A schematic

representation of the extraction step of the polar (unsaponifiable) fraction of vegetable oils is shown in Figure 1.

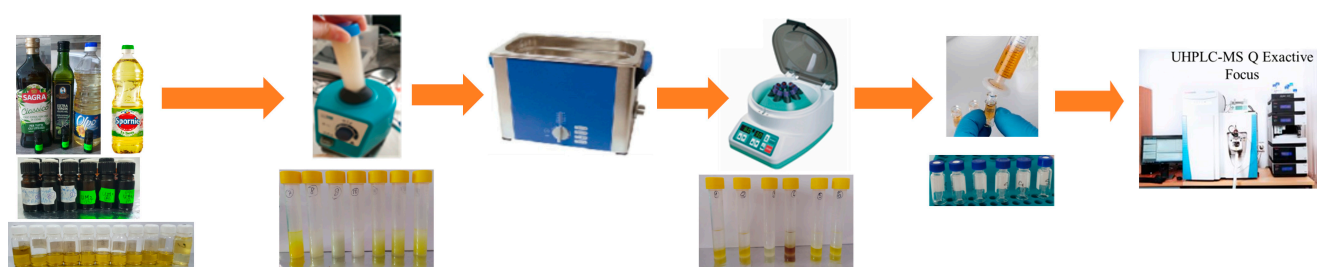


Figure 1. Schematic representation of the extraction of polar fraction from vegetable oils before analysis by UHPLC-MS/MS.

2.4. UHPLC-HRMS Analysis of Phenolic Compounds in Oil Samples

The determination of phenolic compounds content was performed by UHPLC-MS/MS with ESI ionization, using a high-resolution mass spectrometer Q Exactive™ Focus Hybrid Quadrupole-Orbitrap (ThermoFisher Scientific, Bremen, Germany) equipped with HESI and coupled to an UltiMate 3000 UHPLC (ThermoFisher Scientific, Bremen, Germany). The method used was reported in a previous paper [18]. Briefly, the chromatographic separation of the phenolic compounds was carried out on a Kinetex® C18 column at 30 °C. The mobile phase A was water with 0.1% formic acid and mobile phase B was methanol with 0.1% formic acid. The flow rate was 0.3–0.4 mL/min. The mass spectrum was registered in negative ionization mode. Ultrapure nitrogen was employed as a collision and auxiliary gas. The applied voltage was 2.5 kV and the capillary temperature was 320 °C. The collision cell energy ranged from 30 eV to 60 eV. The data were acquired and processed using the Xcalibur software package (Version 4.1). A calibration curve for each phenolic compound was achieved between 0 and 1000 $\mu\text{g} \times \text{L}^{-1}$. The standard solutions of each concentration were obtained from the stock solution of 10 $\text{mg} \times \text{L}^{-1}$ by successive dilutions with methanol. The phenolic acids and flavonoids detected and quantified by the UHPLC method are the following: apigenin, chrysin, galangin, kaempferol, pinocembrin, isorhamnetin, abscisic acid, gallic acid, syringic acid, p-coumaric acid, vanillic acid, caffeic acid, ferulic acid, 3,4-dihydroxybenzoic acid, chlorogenic acid, ellagic acid, p-hydroxybenzoic acid, t-cinnamic acid, (-)-epicatechin, and (+)-catechin.

2.5. Data Analysis

All the UHPLC-HRMS analyses were performed in triplicate. Statistical variances among vegetable oil phenolic contents were tested using Pearson's correlation test at a 0.05 significance level. Principal component analysis (PCA), hierarchical cluster analysis (HCA) and partial least-squares discriminant analysis (PLS-DA) were carried out using a data matrix, including 19 rows corresponding to the investigated oil samples and 25 variables corresponding to phenolic compounds levels obtained from HRMS analysis. The methods were used for the discrimination and classification of the samples. The same methods were applied in the case of adulterated olive oils with corn oil and the data matrix includes 11 rows (pure and adulterated samples) and 25 columns (phenolic compounds levels obtained from HRMS analysis). All the data analyses were carried out using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) and XLSTAT version 15.5.03.3707 (Addinsoft, New York, NY, USA).

3. Results

3.1. Identification of Phenolic Compounds in Vegetable Oils by UHPLC-HRMS

The phenolic compounds were identified in analyzed oil by comparing the retention times with the retention times obtained for the standard compounds, and also by identification of the molecular ion and the corresponding fragments resulting from ionization in negative mode (Table 2). The identification of the main phenolic acids and flavonoids in extra virgin olive oil is presented in Figure 2. In addition to the quantified compounds, other major compounds such as tyrosol, oleocanthal (p-HPEA-EDA), luteolin, but also the aglycone oleuropein mono-aldehyde (3,4-DHPEA-EA) were putatively identified in the extract resulting from EVOO based on the detection of the deprotonated molecular ion $[M - H]^-$ and its characteristic fragments (Figure 3).

Table 2. Identification of phenolic compounds in vegetable oils by UHPLC-MS/MS.

#	Compound	Retention Time [min]	m/z $[M - H]^-$	Mass Fragments	R ²
Phenolic Acids					
1	Gallic acid	0.68	169.0133	125.0231	0.9837
2	3,4-dihydroxybenzoic acid	1.59	153.0183	109.0281	0.9991
3	p-hydroxybenzoic acid	5.40	137.0232	93.0331	0.9996
4	Chlorogenic acid	7.55	353.0879	191.0553	0.9945
5	Syringic acid	8.03	197.0450	182.0212, 166.9976, 153.0547, 138.0311, 123.0075	0.9872
6	Caffeic acid	8.08	179.0338	135.044	0.9986
7	p-coumaric acid	8.59	163.0392	119.0489	0.9976
8	Ferulic acid	8.83	193.0500	178.0262, 134.0361	0.9989
9	Ellagic acid	9.66	300.9990	300.9990	0.9796
10	Cinnamic acid	10.45	147.0441	119.0489, 103.0387	0.9951
11	Abscisic acid	10.04	263.1288	179.9803, 191.9454	0.9995
Flavonoids					
12	(+)-catechin	7.57	289.0719	109.0282, 125.0232, 137.0232, 151.0390, 203.0708, 245.0817	0.9963
13	(-)-epicatechin	8.05	289.0719	109.0282, 125.0232, 137.0232, 151.0390, 203.0708, 245.0817	0.9949
14	Quercetin	10.74	301.0356	151.0226, 178.9977, 121.0282, 107.0125	0.9188
15	Naringin	9.25	579.1718	363.0721	0.9997
16	Hesperidin	9.37	609.1824	377.0876	0.9988
17	Rutin	9.43	609.1462	3345.0614	0.9965
18	Kaempferol	11.62	285.0406	151.0389, 117.0180	0.9916
19	Isorhamnetin	11.80	315.0512	300.0276	0.9637
20	Apigenin	11.86	269.0457	117.0333, 151.0027, 107.0126	0.9977
21	Pinocembrin	12.70	255.0663	213.0551, 151.0026, 107.0125	0.9897
22	Chrysin	13.52	253.0506	143.0491, 145.0284, 107.0125, 209.0603, 63.0226, 65.0019	0.9999
23	Galangin	13.77	269.0458	169.0650, 143.0491	0.9889
24	Pinostrobin	14.84	269.081	179.0554	0.9833
Stilbenes					
25	<i>t</i> -Resveratrol	9.55	227.0707	185.0813, 143.0337	0.9988

R²—coefficient of determination.

3.2. Quantification of Phenolic Compounds in Vegetable Oils

The quantitative data resulting from the investigation of phenolic compounds in vegetable oils indicated that the main phenolic acids identified are p-coumaric, ferulic, ellagic, abscisic, and cinnamic acids, their content varying depending on the type of vegetable oil (Figure 4). As can be seen, walnut oil (W) and extra virgin olive oil (EVOO) have a higher content of ellagic acid (2.4 and 1.2 mg/kg, respectively), while sesame seed oil has a higher content of ferulic acid (2.7 mg/kg) compared to the other types of vegetable oils. EVOO has a cinnamic acid content of 2.2 mg/kg (mean value), a much higher value compared to the other oils—not detected (n.d.)—0.4 mg/kg, thus being a representative phenolic marker for EVOO. Among the quantified flavonoids, pinostrobin,

apigenin, quercetin, and isorhamnetin are the majority, with values ranging between n.d.—5.1 mg/kg for pinostrobin, n.d.—1.7 mg/kg for apigenin, n.d.—1.1 mg/kg for quercetin, and n.d.—0.5 mg/kg for isorhamnetin, respectively.

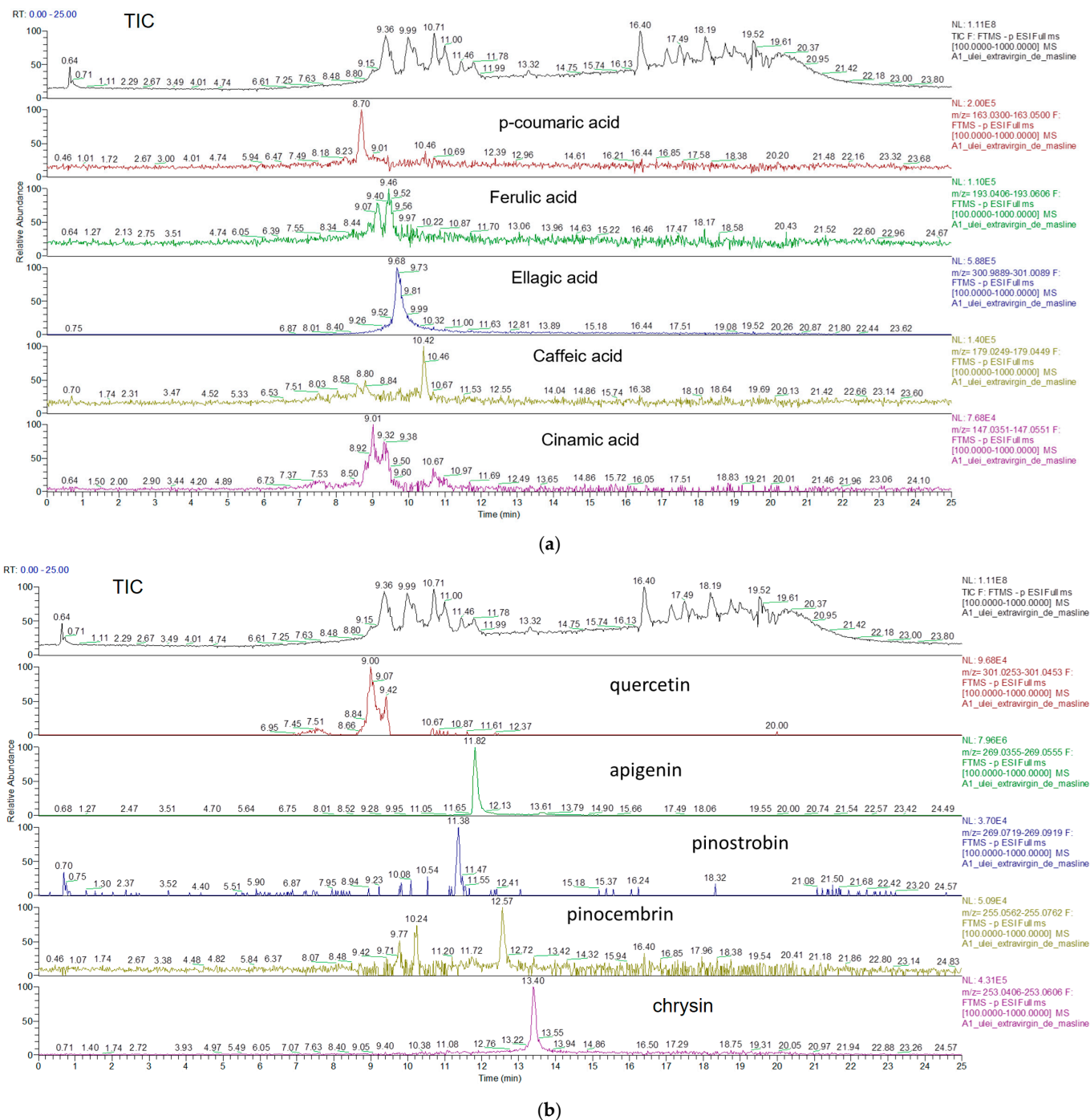


Figure 2. Identification of phenolic compounds in the liquid extract of extra virgin olive oil by UHPLC-MS/MS, negative ionization: (a) phenolic acids, (b) flavonoids.

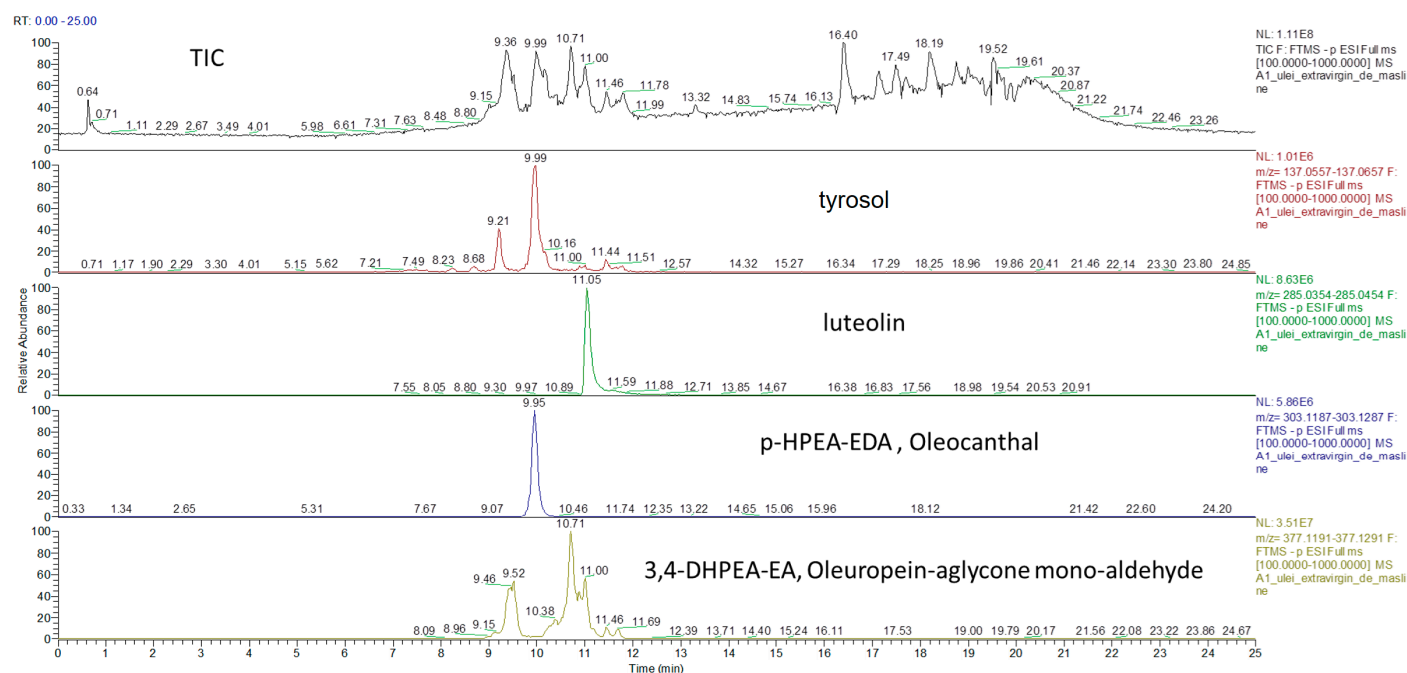


Figure 3. Putative identification of representative phenolic compounds from the liquid extract of extra virgin olive oil by UHPLC–MS/MS, negative ionization.

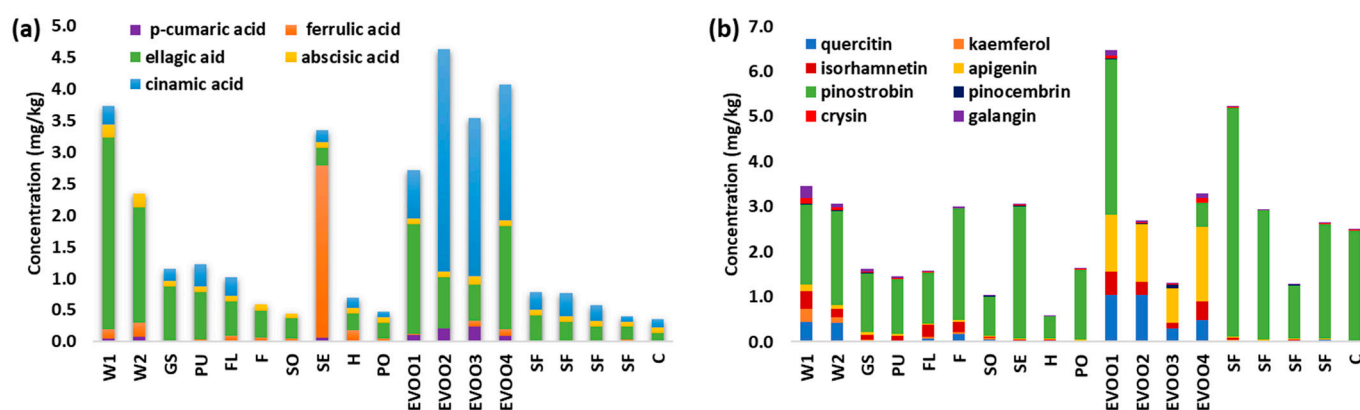


Figure 4. UHPLC-MS/MS profile of phenolic acids (a) and flavonoids (b) in vegetable oils (extra virgin olive oil (EVOO), walnut oil (W), grape seed oil (GS), pumpkin seed oil (PU), flax-seed oil (F), soybean oil (SO), sesame oil (SE), hemp oil (H), poppy seed oil (PO), sun-flower oil (SF), corn oil (C)).

3.3. Discrimination of Vegetable Oils Based on Targeted and Untargeted HRMS Analysis of Phenolic Compounds

For the exploratory analysis of the data from the quantitative analysis of phenolic compounds, principal component analysis (PCA) was performed. The first two principal components (PC 1 and PC 2) with 53.64% of the total variance were extracted for analysis. The distribution of vegetable oils in the PC1-PC2 score plot is shown in Figure 5, where a clear discrimination of extra virgin olive oil and walnut oil from the rest of the oils is observed.

The results indicate that the phenolic markers specific to extra virgin olive oil are the phenolic acids coumaric and cinnamic acids, as well as flavonoids as quercetin, isorhamnetin, apigenin, (+)-catechin, and pinocembrin. For the walnut oil, the phenolic markers are chlorogenic, p-hydroxybenzoic, and abscisic acids (phenolic acids), and also rutin, galangin, kaempferol, and hesperidin (flavonoids).

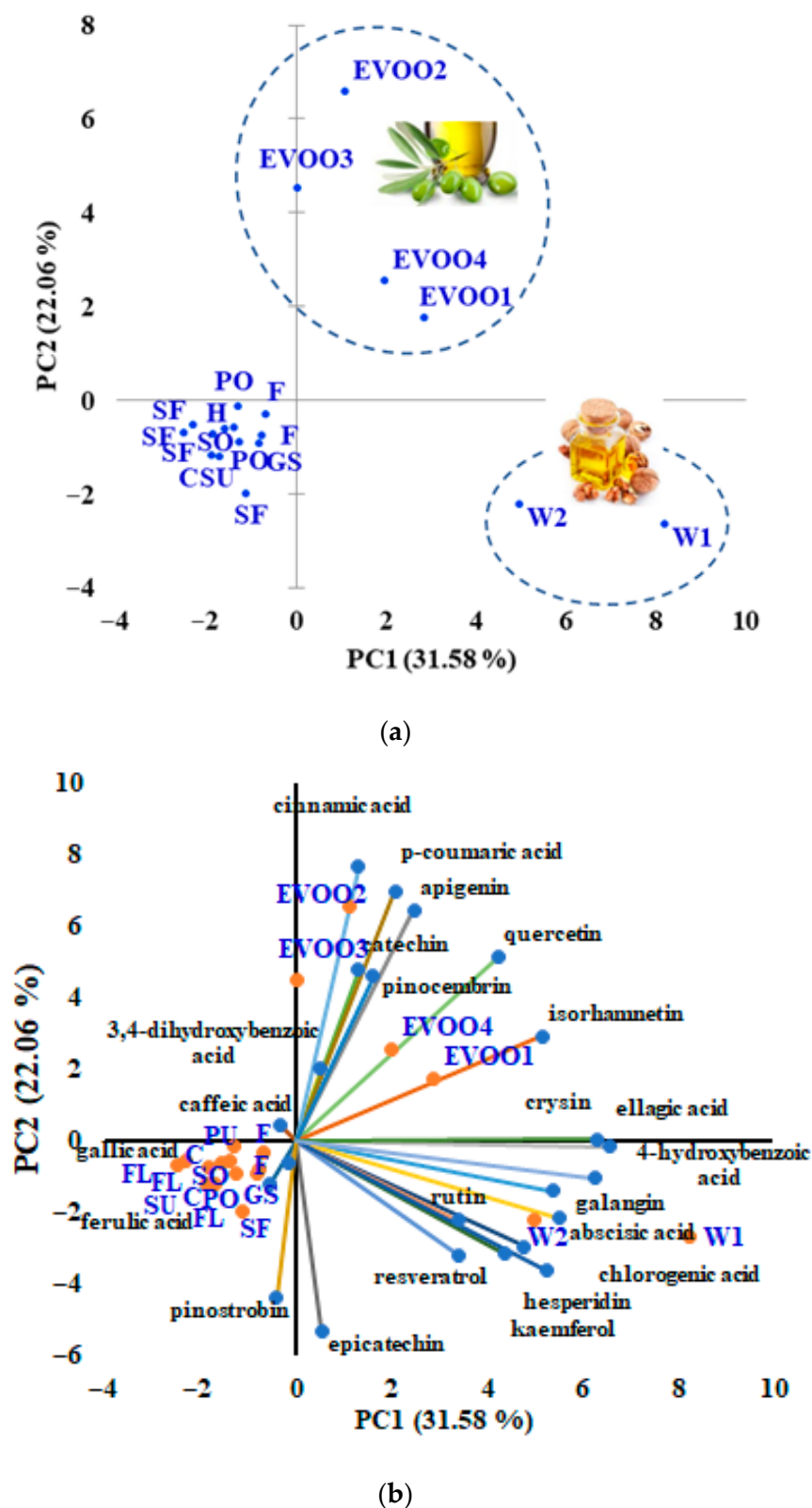


Figure 5. PCA results-scores (a) and loading biplots (b) of vegetable oils based on phenolic compounds profile (extra virgin olive oil (EVOO), walnut oil (W), grape seed oil (GS), pumpkin seed oil (PU), flaxseed oil (F), soybean oil (SO), sesame oil (SE), hemp oil (H), poppy seed oil (PO), sunflower oil (SF), corn oil (C); PC1—first principal component; PC2—second principal component. The points represent mean values of triplicate analysis values.

Hierarchical cluster analysis (HCA) based on quantitative data on phenolic compounds in vegetable oils grouped the investigated oils into four clusters: cluster C1, which groups

most of the investigated olive oils; cluster C2, in which soybean, hemp, flaxseed, grape seed, and pumpkin oils are grouped; cluster C3, in which nut oils and a sample of extra virgin olive oil from Bulgaria are grouped; and cluster C4, in which sunflower, sesame, and corn oils are grouped (Figure 6). As can be seen, based on the minority phenolic compounds, there is a very clear differentiation of extra virgin olive oil from other vegetable oils, being grouped in a well-defined cluster. Vegetable oils obtained from soybeans and seeds (hemp, poppy, flaxseed, and grape seeds) present a similar profile of phenolic compounds, being different from that of vegetable oils obtained from sesame, sunflower, and corn.

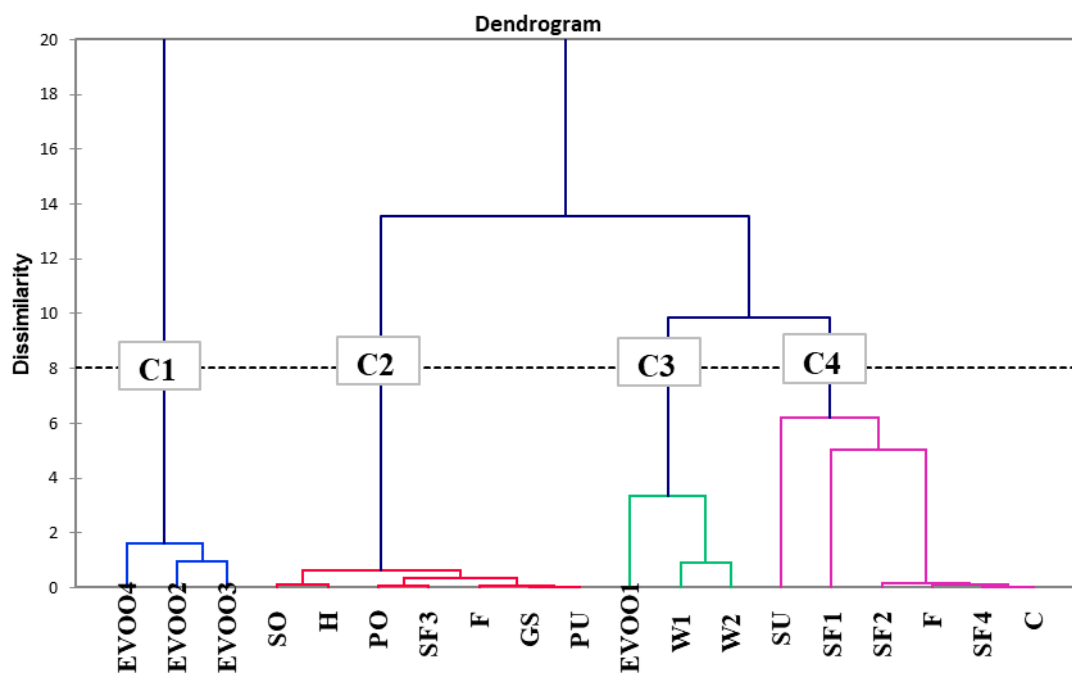


Figure 6. HCA of vegetable oils based on phenolic compounds: extra virgin olive oil (EVOO), walnut oil (W), grape seed oil (GS), pumpkin seed oil (PU), flaxseed oil (F), soybean oil (SO), sesame oil (SE), hemp oil (H), poppy seed oil (PO), sunflower oil (SF), corn oil (C).

Concluding the data on the phenolic compounds profile of different vegetable oils, it can be stated that extra virgin olive oil presents a very different composition, with some similarities to unrefined walnut, sunflower, and corn oils. These similarities regarding phenolic composition suggest that the unrefined oils can be used as adulterants of extra virgin olive oil, by adding them, in different percentages, to extra virgin olive oil.

Following the PCA of the quantitative data on phenolic compounds from oil samples resulting from mixing EVOO1 with different percentages of corn oil (A2—0.5%, A3—1%, A4—2.5%, A5—3%, A6—5%, A7—7.5%, A8—10%, A9—19.5%, A10—50%), the differentiation of EVOO samples adulterated with corn oil is observed, especially adulterated EVOO containing over 3% corn oil (A5–A10), being located on the left side of the PC1 axis. Adulterated EVOO samples with lower percentages of corn oil (0.5%, 1% and 2.5%—A2, A3 and A4) are grouped on the right side of the PC1 axis, alongside unadulterated extra virgin olive oil. The main phenolic markers that underlie the differentiation of the pure EVOO sample from adulterated ones are ellagic and caffeic acids, but also flavonoids such as galangin, chrysin, and pinocembrin (Figure 7).

Thus, based on the experiment of controlled adulteration of EVOO with different percentages of corn oil, it can be concluded that the profile of the investigated phenolic compounds allowed the differentiation of EVOO adulterated with more than 2.5% corn oil.

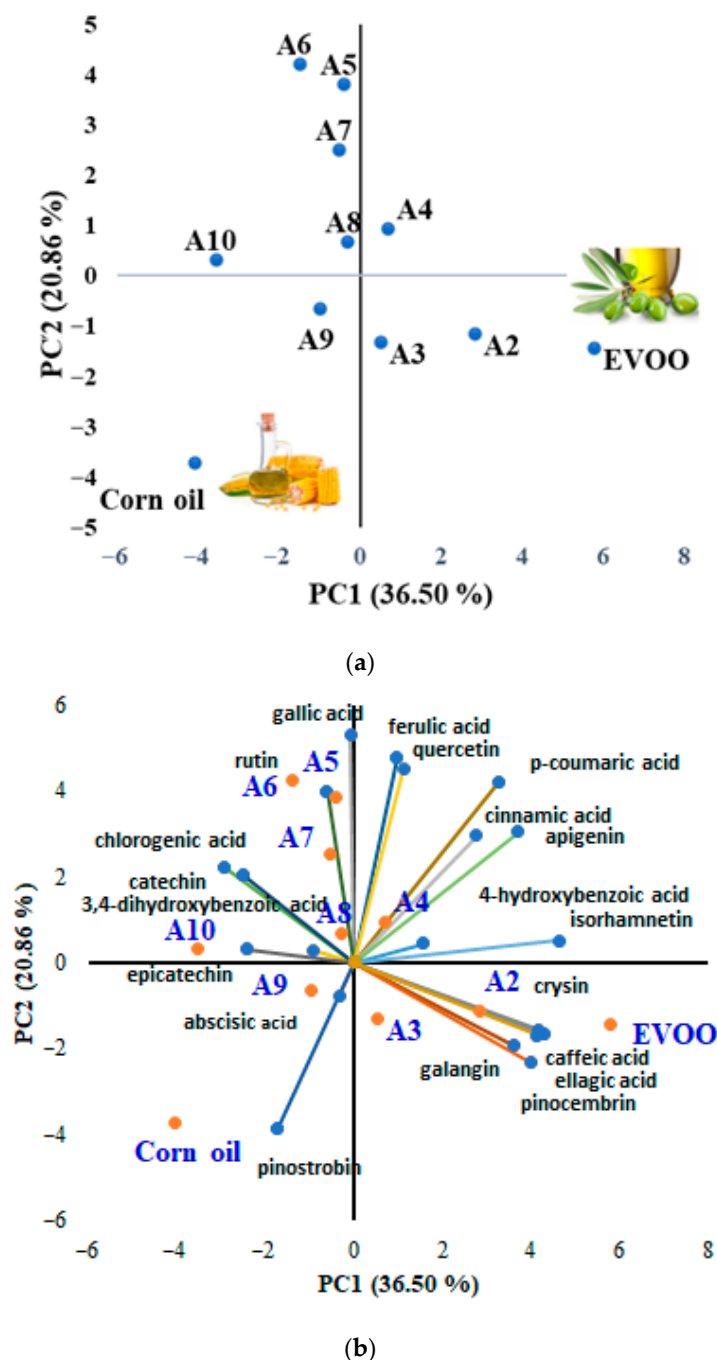


Figure 7. PCA results-scores (a) and loading biplots (b) of extra virgin olive oil samples adulterated with different percentages of corn oil; PC1—first principal component; PC2—second principal component. The points represent mean values of triplicate analysis values. Samples: Corn oil (CO), extra virgin olive oil (EVOO), A2—EVOO adulterated with 0.5% CO, A3—EVOO adulterated with 1% CO, A4—EVOO adulterated with 2.5% CO, A5—EVOO adulterated with 3% CO, A6—EVOO adulterated with 5% CO, A7—EVOO adulterated with 7.5% CO, A8—EVOO adulterated with 10% CO, A9—EVOO adulterated with 19.5% CO, A10—EVOO adulterated with 50% CO.

A partial least-squares discriminant analysis (PLS-DA) model was built to classify the genuine EVOO and adulterated EVOO samples—corn oil—at different concentration levels of adulterant. In PLS-DA binary models, classes are expressed as PLS dummy variables (0 for the genuine class and 1 for the adulterated class). Before, the PLS predicted value of each sample was used for its classification into one class or the other according to a classification threshold (predicted value = 0.5). PLS-DA models were calibrated by full cross-validation

(leave one out), selecting the optimal number of latent variables in accordance with the lowest Root Mean Squared Error of Cross Validation [49]. The correct classification of one sample means the inclusion of it in the right class, as is previously established. In this case, an EVOO sample is correctly classified if it is assigned to the genuine EVOO class. The adulterated EVOO with corn oil samples are correctly classified if these are assigned to the adulterated EVOO class. The corn oil sample is correctly classified if it is not assigned to the genuine EVOO class or adulterated EVOO class. The same situation is for other oil samples (walnut oil, grape seed oil, pumpkin seed oil, flaxseed oil, soybean oil, sesame oil, hemp oil, poppy seed oil, sunflower oil), where these are not assigned by the PLS-DA model to the genuine EVOO class or adulterated EVOO class. PLS-DA confusion matrix of the data obtained from the chromatographic analysis of the samples of extra virgin olive oils adulterated with corn oils led to the results included in Table 3.

Table 3. Sample classification table by PLS-DA.

Model	Analyzed Sample	Correctly Classified Samples/%	Misclassified Samples/%
Genuine EVOO/Adulterated EVOO	EVOO1	100	0
	EVOO2	100	0
	EVOO3	100	0
	EVOO4	100	0
	C/EVOO1 0.5%	83.333	16.667
	C/EVOO1 1%	92.361	7.639
	C/EVOO1 2.5%	100	0
	C/EVOO1 3%	100	0
	C/EVOO1 5%	100	0
	C/EVOO1 7.5%	100	0
	C/EVOO1 10%	100	0
	C/EVOO1 19.5%	100	0
	C/EVOO1 50%	100	0
	C	100	0
	W	100	0
	GS	100	0
	PU	100	0
	F	100	0
	SO	100	0
	SE	100	0
	H	100	0
	PO	100	0
	SF	100	0

EVOO—extra virgin olive oil; C—corn oil; W—walnut oil; GS—grape seed oil; PU—pumpkin seed oil; F—flaxseed oil; SO—soybean oil; SE—sesame oil; HE—hemp oil; PO—poppy seed oil; SF—sunflower oil.

Using this PLS-DA model, the results showed that all the other vegetable oils are correctly classified into a different class, not the EVOO class or adulterated EVOO class, and therefore were 100% correctly classified. Furthermore, the PLS-DA model demonstrates that there is a correct classification of oil samples according to the degree of adulteration if the adulterant concentration (corn oil) is 1%, with accuracy of more than 90%. The 100% classification accuracy is obtained if percentage of adulterant is greater than 2.5%. The

performance of the method developed in this study is comparable with the results reported in the literature.

Ultra-high-performance liquid chromatography (UHPLC) with charged aerosol detection data and PCA was employed to detect adulteration of EVOO with cheaper vegetable and seed oils (soybean, rapeseed, high-oleic sunflower, canola, high-oleic safflower). The method could detect the adulteration of EVOO at a level of 5% for high-oleic sunflower and grapeseed oils and 10% for soybean, canola, and high-oleic safflower oils [50]. In another study, application of ultra-performance convergence chromatography-quadrupole time-of-flight mass spectrometry together with data analysis in adulteration of EVOO with soybean, corn, or sunflower oils was reported. Based on triacylglycerol compositions, the adulterated samples at 0.5% level could be discriminated from genuine oil [51]. Ultra-high-performance liquid chromatography-quadrupole-time of flight-tandem mass spectrometry coupled with PCA could be used to identify adulteration of EVOO with rapeseed, soybean, or camellia oils at a lower concentration of 2% [52]. High-performance liquid chromatography method with ultraviolet detection fingerprinting was applied in the detection of adulteration of Arbequina EVOO with refined olive oil and sunflower oil. The PLS analysis has shown a global quantitative error below 2.9% for a minimum of 2.5% adulterants [53].

The chromatography-based method offers high selectivity and also provides the concentration of adulterants in the olive oil. However, one major disadvantage of this method is the necessity for specific equipment and expertise. Furthermore, chromatography-based methods involve sample preparation steps, including extraction, which is time-consuming and labor-intensive.

4. Conclusions

This work demonstrates that data obtained by UHPLC-MS/MS for the determination of phenolic compounds from different vegetable oil samples are characteristic of the different oils. For instance, walnut oil and extra virgin olive oil present a higher content of ellagic acid (2.4 and 1.2 mg/kg, respectively), while sesame seed oil presents a higher content of ferulic acid (2.7 mg/kg), compared to other vegetable oils. Principal component analysis (PCA), hierarchical cluster analysis (HCA), and partial least-squares discriminant analysis (PLS-DA) were successfully applied to discriminate and classify oil samples in agreement with their botanical origin and to classify the extra virgin olive samples adulterated with different amounts of corn oil. The methodology could be optimized and extended to analyze the adulteration of extra virgin olive oils with other vegetable oils. In order to increase the capacity of the chromatographic method to discriminate adulterated EVOOs, future works could include more data, such as fatty acids, sterols, and pigment levels or the entire chromatograms.

Author Contributions: Conceptualization, E.-I.G. and C.A.; methodology, E.-I.G., I.M.A. and C.A.; software, E.-I.G. and C.A.; validation, E.-I.G., I.M.A. and C.A.; formal analysis, E.-I.G. and C.A.; investigation, E.-I.G., I.M.A. and C.A.; resources, C.A.; data curation, E.-I.G., I.M.A. and C.A.; writing—original draft preparation, E.-I.G. and I.M.A.; writing—review and editing, E.-I.G., I.M.A. and C.A.; visualization, C.A.; supervision, C.A.; project administration, C.A.; funding acquisition, C.A. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a grant from the Romanian Ministry of Education and Research, CNCS—UEFISCDI, project number PN-III-P4-ID-PCE-2020-0923, within PNCDI III.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors gratefully acknowledge the Romanian Ministry of Research, Innovation and Digitization through the NUCLEU Program, Contract no. 20N/05.01.2023, Project PN 23 15 03 01: “Implementation of integrated isotopic-chemical-nuclear analytical methodologies for the authentication of traditional Romanian food products,” for supporting the analytical infrastructure that made this research possible.

Conflicts of Interest: The authors declare no conflicts of interest.

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