agriculture

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Flavouring Extra-Virgin Olive Oil with Aromatic and Medicinal Plants Essential Oils Stabilizes Oleic Acid Composition during Photo-Oxidative Stress

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Citation: Barreca, S.; La Bella, S.; Maggio, A.; Licata, M.; Buscemi, S.; Leto, C.; Pace, A.; Tuttolomondo, T. Flavouring Extra-Virgin Olive Oil with Aromatic and Medicinal Plants Essential Oils Stabilizes Oleic Acid Composition during Photo-Oxidative Stress. *Agriculture* **2021**, *11*, 266. https://doi.org/10.3390/agriculture11030266

Academic Editor: Silvia Tavarini

Received: 19 February 2021
Accepted: 17 March 2021
Published: 20 March 2021

Abstract: Essential oils (EOs) from medicinal and aromatic plants (MAPs) are well-known as natural antioxidants. Their addition to extra-virgin olive oil (EVOO) can contribute to reducing fat oxidation. The main aim of this study was to improve both food shelf-life and aromatic flavour of EVOO, adding different EOs of Sicilian accessions of common sage, oregano, rosemary and thyme. The morphological and production characteristics of 40 accessions of MAPs were preliminarily assessed. EOs from the most promising accessions of MAPs were analysed by gas-chromatography and mass spectrometry. Photo-oxidative studies of the EOs were carried out and the determination of the EVOO fatty acids obtained from 4 Italian olive varieties was also made. EO content was on average 1.45% (*v*/*w*) for common sage, 3.97% for oregano, 1.42% for rosemary and 5.90% for thyme accessions. The highest average EO yield was found in thyme (172.70 kg ha^{-1}) whilst the lowest (9.30 kg ha^{-1}) in rosemary accessions. The chemical composition of EOs was very different in the four MAPs in the study. No significant change of oleic acid percentage was detected in the mixture of EVOO with EO samples. The results seem to highlight the presence of an antioxidant effect of EOs on EVOO.

Keywords: extra-virgin olive oil; aromatic and medicinal plants; essential oil; gas-chromatography and mass spectrometry analyses; antioxidant; oleic acid

1. Introduction

Extra-virgin olive oil (EVOO), an appreciated food especially in the countries of the Mediterranean area, in recent years has gained interest among the consumers in North America and Northern Europe, especially flavoured with spices, herbs or fruits. The production of flavoured olive oils is a traditional practice in Mediterranean area, in order to enhance the sensorial characteristics of the original olive oils and to improve the sensory properties of the foods [1,2]. The success of these innovative products is associated with various health benefits as well as with the taste and flavour. Flavoured olive oil can represent an extremely interesting product for oil and aromatic plant producers to diversify their offer.

The numerous health benefits of EVOO depend on its particular composition [3,4]. It is composed primarily of triacylglycerols (around 97.00–98.00%), minor amounts of free

fatty acids and glyceridic compounds, phospholipids and oxidized triacylglycerols and around 1% of unsaponifiable constituents of varied structure and polarity. There are a high proportion of monounsaturated fatty acids, mainly oleic acid, and a modest presence of polyunsaturated fatty acids. In addition, some natural antioxidants such as tocopherols, carotenoids, sterols and phenolic compounds are present. Some health properties of EVOO depend on content of oleic acid that serves to slow down penetration of fatty acids into arterial walls. The EVOO stability rate depends on both antioxidant compounds and storage conditions. During storage nutritional and health properties of extra-virgin oil may be changed by oxidative phenomena. The addition of naturally occurring antioxidants causes an improvement on the shelf life and nutritional value of oil.

Recently a study has showed that the initial quality of EVOO enriched and not enriched with lycopene commercially available prevails over the slight antioxidant activity that lycopene could exert. The EVOO composition in the main and minor components is the key factor that determines its performance in gastrointestinal conditions [5]. The methods commonly used to flavour olive oils are different [6]. The "gourmet oils" are prepared left herbs, spices and fruits mixed with oil at room temperature for a defined time. The mixture is, then, filtered to remove turbidity and solid parts. Infusion is the oldest method of oil aromatization and the most considered from the producers [7]. On the other hand, comilling the olives with herbs, spices or fruits such as lemons and bergamots during the oil productive process is a new approach for preparing clear and safe flavoured olive oils. Another method is the ultrasound-assisted maceration.

The flavouring technique significantly influences the chemical and sensory quality of the olive oils. In particular, the infusion of oils with spices caused a greater oxidative degradation due to lower content of total phenols. On the other hand, the olive oils obtained by combined malaxation of olives and spices were less bitter. The aromatic quality was not significantly affected by the method of flavouring, except for sulphur compounds that were greater in oils obtained by malaxation [8].

Rarely, the addition of essential oils (EOs) of medicinal and aromatic plants (MAPs) is used to obtain flavoured olive oils. These plants have drawn more attention due to the antimicrobial, antifungal, insecticidal and antioxidant effects [9–11]. Application of antioxidants is one of the technically simplest ways of reducing fat oxidation. Natural antioxidants from edible aromatic plants have many advantages such as (a) to be accepted by consumers, (b) to be considered safe, (c) to come from natural resources and (d) to have functional and sensory properties. Considering the beneficial effects of fatty acid contained in olive oils, addition of the MAPs EOs could represent a useful technique to preserve fatty acid profile in the EVOO composition. The results of preliminary studies showed that the presence of oregano EO, preserve sensory quality of extra virgin olive oil prolonging the shelf life of this product [12].

In Sicily (Italy), MAPs are widely present in the native flora. The great diversity in climate, soil and habitat of this Mediterranean region has contributed and provided good plant genetic resources. As reported in numerous studies [13–17], the exploitation of MAPs biodiversity and the cultivation of native accessions under open field conditions highlighted the effects of genetic and environmental factors on the qualitative and quantitative production of the EOs [18]. Furthermore, when analysing the EO profile of various aromatic species, specific Sicilian chemotypes were found. Following these findings, a number of Sicilian MAPs have now been distinguished from the same species growing in other Mediterranean regions, thus making their EOs of particular interest due to specific compounds and aroma.

In this study, to keep the biological properties of EVOO unaltered and improve them in long-term period, it was then decided to investigate the effect of adding EOs of MAPs to prevent oxidative processes. Native accessions of common sage, oregano, rosemary and thyme were used for obtaining EOs. Among different MAPs found in Sicilian areas, these species were chosen as they were widely used as a condiment in the Mediterranean diet and had already been used for the production of flavoured oils.

Thus, the aims of this study were: (i) to select the Sicilian accessions of common sage, oregano, rosemary and thyme, grown in collection fields, considering the best ratio between EO content and plant dry weight and (ii) to test the efficacy of the different EOs of these species as a natural antioxidant to improve both the shelf life and the quality of EVOO.

2. Materials and Methods

2.1. Experimental Site of MAPs

A number of Sicilian native accessions of common sage (*Salvia officinalis* L.), oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart), rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymbra capitata* (L.) Cav.) were grown in 4 separate collection fields (one for species) at the "Orleans" experimental farm (Palermo, 31 m a.s.l., 38°06′26.2″ N, 13°20′56.0″ E) belonging to the University of Palermo, north-west Sicily. These accessions had been previously collected in various areas of Sicily and subsequently subjected to taxonomic characterization using analytical keys and comparing them to exsiccata stored at the Botanical Gardens of the University of Palermo.

Soil at "Orleans" experimental area was sandy clay loam (56% sand, 23% clay, 21% silt) with a pH of 7.91, 19 g kg^{-1} organic carbon, 58 g kg^{-1} total carbonates, 37 g kg^{-1} active carbonates, 13.2 g kg^{-1} total nitrogen, 18.11 mg kg^{-1} assimilable phosphorus and 320 mg kg^{-1} exchangeable potassium.

The climate of the area is Mediterranean with mild, humid winters and hot, dry summers [19]. The average annual temperature is 18.40 °C, with average minimum and maximum temperatures of 14.80 and 21.70 °C, respectively. Annual average rainfall is approx. 600 mm.

2.2. Collection Fields of MAPs and Main Cultivation Practices

In 2018, a total of 40 accessions of Sicilian MAPs (5 for common sage, 15 for oregano, 10 for rosemary and 10 for thyme) were assessed at the 4 collection fields (Figure 1).

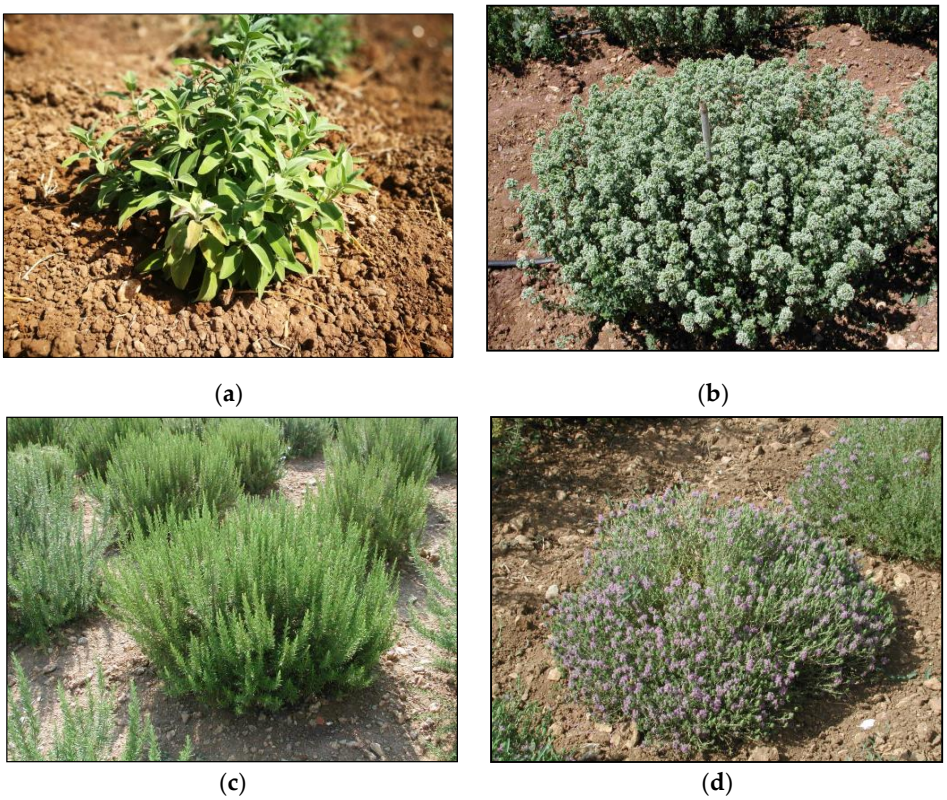

Figure 1. Accessions of medicinal and aromatic plants (MAPs) grown in the collection fields. (**a**) refers to common sage, (**b**) referes to oregano, (**c**) refers to rosemary and (**d**) refers to thyme plants.

Common sage and rosemary were planted at 2 m × 1 m spacing while oregano and thyme at 1.5 m × 1 m spacing. The accessions had previously been collected in various Sicilian areas which were different for soil and climate conditions. Plants were 4 years old. Each collection field was equipped with a drip irrigation system. However, plants were grown in dry conditions, this being a traditional practice used for cultivation of MAPs in Sicily. Plants exploited the residual soil fertility and no chemical fertilization application was given during the year. Weed control was manual and no pesticides were used. Harvest was carried out when most plants of the 4 species were at the full blooming stage.

2.3. Morphological and Production Parameters

Morphological and production measurements were made on a sample plot of 10 plants per accession, excluding the border rows.

The main morphological parameters (data not shown) of each species, such as plant height, number of branches, number of stems, floral spike length, flowers length and width were recorded. The plant fresh weight of above and below-ground plant parts was determined by harvesting the plants. The harvested plant material was, subsequently, dried in an oven at 65 °C for 48 h. The plant dry weight was, then, calculated. Dry matter yield per hectare was also estimated.

Samples of leaves and flowers were collected from the accessions of each species when 80% of the plants were in full flowering stage. These samples were, subsequently, hydrodistilled for 2 h in a Clevenger-type apparatus with a separated extraction chamber. EOs obtained were dried over anhydrous sodium sulphate and stored in dark flask at −18 °C in freezer until the EO samples were analysed by gas–liquid chromatography (GC) and mass spectrometry (MS) or used in the studies. EO yield was calculated by multiplying dry matter yield by oil content, by 0.90 (approximate specific gravity of oil) [20–22].

Finally, for each accession within the 4 species, the EO content was related to dry weight in order to select the most promising accessions.

2.4. Chemicals

Potassium hydroxide and solvents were purchased from Sigma Aldrich (Steinhem, Germany) (analytical grade) hexanal. (E)-2-hexenal and 2-methyl-pentanol were purchased from Sigma (Steinhem, Germany). Activated charcoal (0.50–1.00 mm; 18–35 mesh ASTM) was purchased from E. Merck (Schuchardt, Germany). The charcoal was cleaned by treatment in a Soxhlet apparatus with diethyl ether and was tested in order to verify the absence of any absorbed substances.

2.5. GC-MS Analyses of EOs

Analyses of EOs were performed by GC-MS Shimadzu QP 2010 plus equipped with an AOC-20i auto-injector (Shimadzu, Kyoto, Japan), a split/split-less injector (t = 280 °C) and a capillary column (30 m, 0.25 mm i.d. 0.25 mL film thickness) coated with DB WAX (polyethylene glycol. JW).

The temperature program was 40 °C for 5 min and from 40 to 250 with a rate of 2 °C min^{-1} and from 250 to 270 with a rate of 10 °C min^{-1}. The temperature of injector was maintained at 250 °C while the temperature of detector was maintained at 280 °C. The compounds were identified by comparing their retention time and mass spectra with published data, Adams, NIST 11, Wiley 9 and FFNSC 2 mass spectral database.

The samples were injected in splitless mode. The quantitative composition was obtained by peak area normalization, and the response factor for each component was considered to equal 1.

2.6. Photo-Oxidative Studies

100 µL of olive oil was transmethylated with 10 mL of KOH (2M) methanol solution according to the European Standard NF EN ISO 5509 (2000). Fatty acid methyl esters (FAME) obtained were transferred in hexane and analysed according to the European

Standard NF EN ISO 5508 (1995). Analyses were performed by using a Shimadzu gas chromatograph (GC) equipped with a split/split-less injector (t = 280 °C) and flame ionization detector (FID) (t = 250 °C). A capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness) coated with DB WAX (polyethylene glycol, JW) was used. The inlet pressure of the hydrogen as carrier gas was 154 kPa with a ratio of 70. The oven temperature program was as follows: 40 °C for 5 min and from 40 to 250 with a rate of 2 °C min^{-1} and from 250 to 270 with a rate of 10 °C min^{-1}. The temperature of injector was maintained at 250 °C while the temperature of detector was maintained at 280 °C. The compounds were identified by comparing their retention time and mass spectra with published data, Adams, NIST 11, Wiley 9 and FFNSC 2 mass spectral database.

In this study, EVOOs of four varieties of olive (*Olea europaea* L.) were considered. In Table 1, the initials of the varieties both in the preveraison (green fruit) and veraison (brown fruit) stages are reported.

Table 1. List of the varieties of olive in two diverse stages with relative initials.

Variety of Olive	Preveraison Stage (Green Fruit)	Veraison Stage (Brown Fruit)
Arbequina	AV	AI
Biancolilla	BV	BI
Cerasuola	CV	CI
Nocellara del Belice	NV	NI

2.7. Fatty Acid Determination before and after Photo-Oxidative Studies

Olive oil in n-hexane was transmethylated with a solution of KOH (2M) according to the European Standard NF EN ISO 5509 (2000). Fatty acid methyl esters (FAME) were analysed according to the European Standard NF EN ISO 5508 (1995). Analyses were performer by using a GC-MS Shimadzu QP 2010 plus equipped with an AOC-20i autoinjector (Shimadzu, Kyoto, Japan), a split/split-less injector (t = 280 °C) and a capillary column (30 m, 0.25 mm i.d. 0.25 mL film thickness) coated with DB WAX (polyethylene glycol. JW) was used. Helium was the carrier gas (1 mL min^{-1}); ionization voltage 70 eV. The temperature was initially kept at 40 °C for 5 min. Then gradually increased to 250 °C at 2 °C min^{-1} rate. Held for 15 min and finally raised to 270 °C at 10 °C min^{-1}. One µL of samples was injected at 250 °C automatically and in the splitless mode; transfer line temperature, 295 °C.

2.8. Statistical Analysis

Statistical analyses were performed using the package MINITAB 17 (State College, PA, USA) for Windows. Data of all production parameters of the MAPs accessions were processed using analysis of variance. The difference between means was carried out using Tukey's test. Concerning percentage content of oleic acid in the EVOO samples and in the mixture of EVOO with EO, all the representative values were shown using mean ± standard error calculation.

3. Results and Discussion

3.1. Agronomic Assessment of Common Sage, Oregano, Rosemary and Thyme Accessions

Data regarding the production parameters of the Sicilian MAPs are shown in Table 2. In the table, the initials CS indicate accessions of common sage, OR of oregano, RSM of rosemary and THY of thyme.

Table 2. Production characteristics of the four MAPs during the study period.

Species	Dry Weight (g plant^{-1})	Dry Matter Yield (kg ha^{-1})	EO Content (% *v*/*w*)	EO Yield (kg ha^{-1})
Common sage				
CS1	134.06 d	671.33 d	1.08 d	6.35 e
CS2	329.53 b	1645.10 b	1.10 cd	16.32 b
CS3	105.53 e	529.07 e	2.04 a	9.47 d
CS4	284.27 b	1423.60 c	1.20 c	15.43 c
CS5	361.30 a	1810.73 a	1.80 b	29.55 a
Oregano				
OR1	770.13 b	5139.44 b	5.82 b	268.51 a
OR2	399.60 k	2666.30 j	6.15 a	147.12 e
OR3	221.73 n	1478.53 m	3.01 k	36.74 n
OR4	504.03 g	3360.72 f	5.11 e	154.72 d
OR5	698.12 c	4652.69 c	5.12 d	218.52 b
OR6	503.93 g	3358.79 f	5.55 c	167.85 c
OR7	468.80 h	3125.88 g	4.02 f	113.12 h
OR8	441.77 i	2952.40 h	3.45 h	91.68 j
OR9	379.93 m	2529.14 l	3.52 g	80.14 k
OR10	413.20 j	2754.71 i	4.01 f	99.41 i
OR11	905.80 a	6039.59 a	2.26 n	122.33 g
OR12	684.12 d	4562.01 d	3.15 i	129.18 f
OR13	521.50 e	3476.67 e	3.11 j	97.30 i
OR14	390.70 l	2604.79 k	2.82 l	65.92 m
OR15	503.07 f	3364.21 f	2.32 m	70.51 l
Rosemary				
RSM1	42.20 h	211.33 h	1.51 b	2.62 h
RSM2	79.23 f	396.51 f	1.21 d	4.20 g
RSM3	274.53 c	1376.77 c	0.78 g	9.65 c
RSM4	339.20 b	1709.11 b	1.01 e	15.37 b
RSM5	534.76 a	2684.33 a	1.52 b	36.61 a
RSM6	47.67 g	240.07 g	2.32 a	5.04 f
RSM7	18.53 j	93.52 j	2.31 a	2.03 i
RSM8	205.63 d	1034.81 d	0.91 f	8.42 d
RSM9	26.20 i	131.61 i	1.42 c	1.66 i
RSM 10	117.23 e	586.43 e	1.21 d	6.29 e
Thyme				
THY1	337.67 i	2252.70 i	4.51 i	91.28 h
THY2	624.50 c	4174.77 c	5.96 d	223.94 c
THY3	416.67 g	2777.27 g	5.20 h	130.26 g
THY4	494.60 f	3294.80 f	6.02 c	178.45 e
THY5	656.17 a	4374.20 a	6.52 b	256.57 a
THY6	626.17 b	4177.20 b	6.80 a	256.51 a
THY7	75.17 j	501.73 j	5.52 g	25.58 i
THY8	381.30 h	2541.93 h	5.78 f	133.24 f
THY9	522.60 e	3486.93 e	5.90 e	185.94 d
THY10	607.20 d	4048.20 d	6.80 a	249.13 b

Means followed by the same letter in the same column are not significantly different for $p \leq 0.05$ according to test of Tukey.

Results of one-way ANOVA revealed significant differences between the accessions within each species for all the production parameters tested.

For common sage, CS5 and CS2 were of considerable interest regarding dry weight, dry matter yield and EO yield. The highest average value of EO content was obtained by CS3 (2.04%), while the lowest value was found in CS1 (1.08%).

In the case of oregano, EO content percentage ranged from 2.26% (OR11) to 6.15% (OR2). Average EO content of the 15 accessions was 4.01%. OR1 (268.51 kg ha^{-1}) and OR5

(218.52 kg ha^{-1}) obtained the highest average EOs yields. Concerning other parameters in the study, OR11 and OR1 performed best among the oregano accessions.

High variability in production parameters was observed also for rosemary accessions. In particular, RSM5 obtained the highest average dry weight (534.36 g), dry matter yield (2684.33 kg ha^{-1}) and EO yield (36.61 kg ha^{-1}); in contrast, lowest average values were found in RSM7. EO percentage content ranged between 2.32% and 0.78%; the highest average values of EO content were recorded by RSM6 and RSM7. It is worth noting that RSM7, in contrast, recorded the lowest values for dry weight, dry matter yield and EO yield.

Observing the data of thyme accessions, THY5 and THY6 performed the best and produced values higher than the average for the field. Among the accessions, THY10 also performed the best for EO content percentage (6.80%).

When considering the ratio between EO content and plant dry weight, it was found to be highly different among accessions of each species (Figure 2).

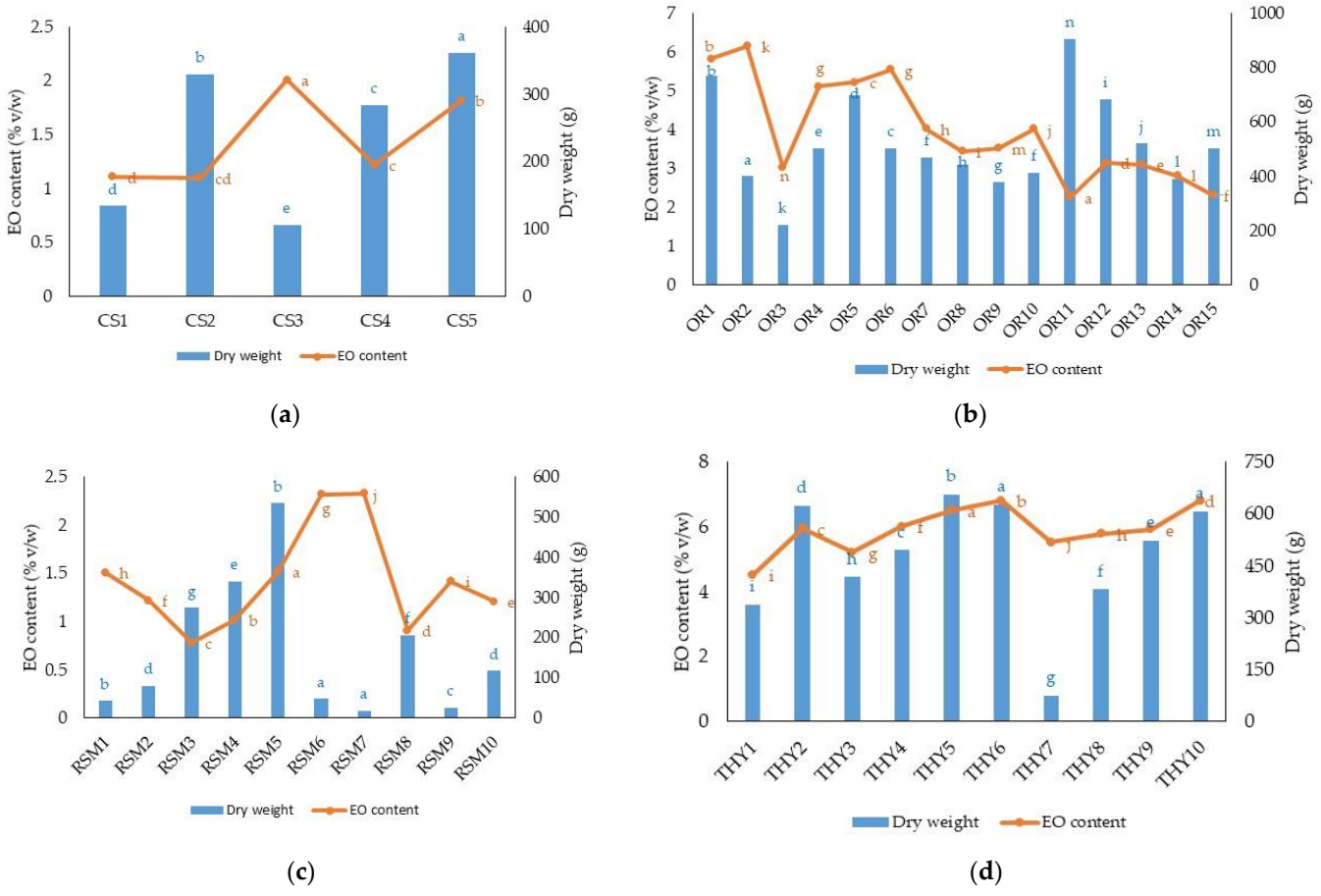

Figure 2. Ratio between essential oils (EO) content and plant dry weight. Means followed by the same letter in the same column are not significantly different for $p \leq 0.05$ according to test of Tukey. Graph (**a**) refers to common sage, graph (**b**) refers to oregano, graph (**c**) refers to rosemary and graph (**d**) refers to thyme.

For common sage, CS3 obtained the best performance while CS2 and CS4 recorded a high average dry weight compared to those of EO content. In the case of oregano, the best ratio between EO content and plant dry weight were found in OR2. On the other hand, OR11 and OR12 showed low average EO content compared to dry weight. For rosemary, RSM7 performed better than other accessions. However, RSM6 also showed high average EO content compared to dry weight. Finally, THY7 was the most promising accession of the thyme accessions. THY2 and THY5, however, were not remarkable in this respect.

In this study, significant differences in terms of yield parameters were found between accessions of each MAP during the test period. These differences mainly depend on the effects of genetic and environmental factors. Various authors [23–27] have reported that genetic variation occurring in native germplasm significantly affects EO content and yield of MAPs. Others [17,26–32] have found that climate factors can greatly influence the performance of MAPs in terms of dry weight, dry matter and essential oil yields. In our study, all accessions of common sage, oregano, rosemary and thyme were assessed under the same climate, soil and growth conditions, thus, the differences in production parameters were due to genotype response to environmental conditions. In the Mediterranean area, literature [33–37] shows different mean values of EO content and yield mainly depending on the climate and soil characteristics of the area and cultivation practices of MAPs. When comparing our findings with those reported in these studies, differences and similarities were found which can be explained by the study of genetic and environmental factors. On the basis of that, it is possible to say that changes in these factors can produce differences in the chemical composition of the EOs in plants of the same species in different environments.

In this study, the ratio between EO content and dry weight yield was also assessed in order to select the most promising accessions within each MAP. High production of EO in relation to dry weight yield is crucial in the cultivation of MAPs. If the EO content is found to be too low compared to plant dry weight, the production process would not be viable for farmers from an economic point of view. On the contrary, when EO content is higher, the production process is, instead, profitable. Therefore, the creation of mixtures of EVOO with MAP EOs requires a preliminary assessment of the EO content and its availability in the long term. This could allow for an estimation of the real cost of this condiment in the market.

3.2. Chemical Composition of the MAPs EOs

The chemical composition of EOs of common sage, oregano, rosemary and thyme accessions are shown in Table 3.

Table 3. Chemical composition of EOs of the common sage, oregano, rosemary and thyme accessions.

RT [a]	KI [b]	Compound	Common Sage	Oregano	Rosemary	Thyme
		Monoterpene Hydrocarbons	27.73	31.31	31.47	28.86
10.22	938	α thujene	0.37	0.79	0.11	0.91
10.60	944	α pinene	4.93	0.90	11.25	1.04
11.59	957	camphene	6.88	t	6.88	0.41
11.92	961	thuja-2.4(10)-diene			0.32	
13.10	974	verbenene			0.16	
13.23	975	sabinene	0.39	0.16	0.05	
13.46	978	ß pinene	8.39	0.01	3.53	0.16
14.61	990	ß-myrcene	3.83	2.24	1.93	2.36
15.39	997	Mentha-1(7),8-diene			0.04	
15.56	999	phellandrene α	0.04	0.36	0.28	0.38
15.66	1000	δ-3-carene		0.11	2.64	
16.34	1012	terpinene α	0.23	3.42	0.35	2.87
16.60	1017	ρ-cymene		0.01	0.03	
16.89	1022	o-cymene	0.22	9.70	3.09	8.73
17.24	1028	sylvestrene	1.65	0.76		0.75
17.99	1042	(Z)-ß-ocimene	0.15	0.02		
18.70	1053	(E)-ß-ocimene	0.02	0.10		0.05
19.37	1064	terpinene γ	0.43	12.59	0.32	10.98
21.32	1094	Mentha-2.4(8)-diene	0.20	0.13	0.40	0.22
21.70	1099	Cymenene			0.09	

Table 3. *Cont.*

RT [a]	KI [b]	Compound	Common Sage	Oregano	Rosemary	Thyme
		Oxygenated Monoterpenes	51.20	64.06	61.87	65.00
17.37	1031	eucalyptol	20.58	0.06	39.38	
20.21	1077	sabinene hydrate cis	0.14	0.64		0.43
22.43	1109	Sabinene hydrate <trans->	0.10	0.12		
22.51	1110	linalool				0.10
22.57	1111	Pinene oxide<α->	0.09	0.36	2.27	0.22
22.79	1114	thujone<cis->	8.25	0.02		
23.62	1125	thujone<trans->	5.78			
23.68	1126	fenchol <exo>			0.05	
24.29	1134	Campholenal α			0.03	
25.14	1144	Pinocarveol trans			0.30	
25.52	1149	camphor	13.77		4.81	
26.29	1158	Eucarvone	0.09			
26.55	1161	pinocamphone trans			0.16	
26.69	1163	pinocarvone			0.60	
27.29	1170	isocitral<(Z)->			0.10	
27.42	1171	Borneol	0.80	0.07	10.40	1.00
27.65	1174	pinocamphone cis			0.50	
28.08	1178	terpinen-4-ol	0.27	0.32	0.46	0.61
29.18	1190	α terpineol	0.09	0.02	0.78	
29.31	1192	dihydro carvone cis				0.06
29.91	1198	verbenone			0.26	
31.68	1228	thymol methyl ether		0.24		
32.27	1238	carvacrol methyl ether		3.70	0.17	
32.68	1245	carvone			0.17	
35.29	1287	Isobornyl acetate	0.04		1.43	
35.54	1291	thymol	1.09	58.40		0.23
36.56	1307	carvacrol		0.11		62.35
39.42	1348	thymol acetate	0.03			
		Sesquiterpene Hydrocarbons	18.17	1.17	5.30	4.13
38.42	1334	elemene<δ>	0.03			
40.80	1367	ylangene α	0.24	0.05		
41.15	1372	copaene α	0.06			
41.64	1379	Bourbonene ß	0.04			
43.16	1398	Longipinene<ß>	1.40			
43.34	1401	Longifolene	0.09			
43.83	1409	caryophyllene (Z)	6.08	0.72	4.91	3.93
44.47	1419	caryophyllene (E)	0.31		0.02	
44.93	1427	Aromadendrene	3.98			
45.52	1436	Barbatene<ß>	0.35			
46.05	1444	caryophyllene α	2.88	0.02	0.37	0.07
46.38	1449	Muurola-3-5-diene cis	0.30			
47.21	1462	Unknown	0.03			
47.42	1465	Cadina-1(6).4-diene cis	0.09	0.08		
47.68	1469	Muurola-4(14).5-diene	0.07			
48.16	1476	Aristolochene<4.5-di-epi->	0.20			
48.39	1480	Muurolene (γ)	0.84			
48.55	1482	bicyclogermacrene	1.06			
49.70	1499	amorphene γ	0.03	0.15		0.13
50.02	1503	amorphene δ	0.09	0.15		
		Other	0.26	0.00	0.67	0.65
14.14	985	octen-3-ol	0.20	t	0.52	0.58
14.40	988	octanone	0.06	t	0.15	
15.36	997	octan-3-ol				0.07
		Total	97.36	96.54	99.31	98.64

[a] Retention time. [b] Kováts retention index.

The chemical composition of EOs was very different in the four MAPs in the study, highlighting high diversity among the species.

The average percentage content of monoterpene hydrocarbons was found, however, to be similar for the various species. It was, in fact, 27.73% in common sage EO, 31.31% in oregano EO, 31.47% in rosemary EO and 28.86% in thyme EO, respectively. Among the monoterpene hydrocarbons, α pinene was found to be one third of the total EO content in rosemary, while γ terpinene represented almost half of the total EOs content in oregano and thyme plants.

Oxygenated monoterpenes were the prevailing group of compounds in EOs of the four species. The average content percentage of this group ranged between 51.20% (common sage EO) and 65.00% (thyme EO). In particular, eucalyptol was found to be the main compound in common sage (20.58%) and rosemary (39.38%) EOs. Analyses of EOs revealed that borneol (10.40%) was more abundant in rosemary EO while its oxidation product, camphor (13.77%), was more represented in common sage EO. In the case of oregano and thyme plants, the main compounds among the oxygenated monoterpenes were thymol (58.40%) and carvacrol (62.35%), respectively.

Concerning the average percentage content of sesquiterpene hydrocarbons, it was found to be different in EOs of the four MAPs. It was 18.17% in common sage EO, 4.13% in oregano EO, 5.30% in rosemary EO and 1.17% in thyme EO, respectively.

3.3. Photo-Oxidative Studies

In order to study the possible employment of EOs as antioxidant to EVOO, the concentration 0.15% v/v of the MAPs EO/EVOO mixtures was chosen. This percentage was the minimum that guaranteed the presence of plant volatile organic compounds (VOCs) in the headspace. An amount of 10 mL of different mixtures were placed in a pyrex tube and irradiated at 360 nm at 40 °C for different time by using an UV test. Oleic acid was the most abundant fatty acid considered in the EVOO samples.

The percentage variation of oleic acid in the oil samples of Cerasuola "green" (CV) after photo-oxidative at different irradiation time is showed in Figure 3.

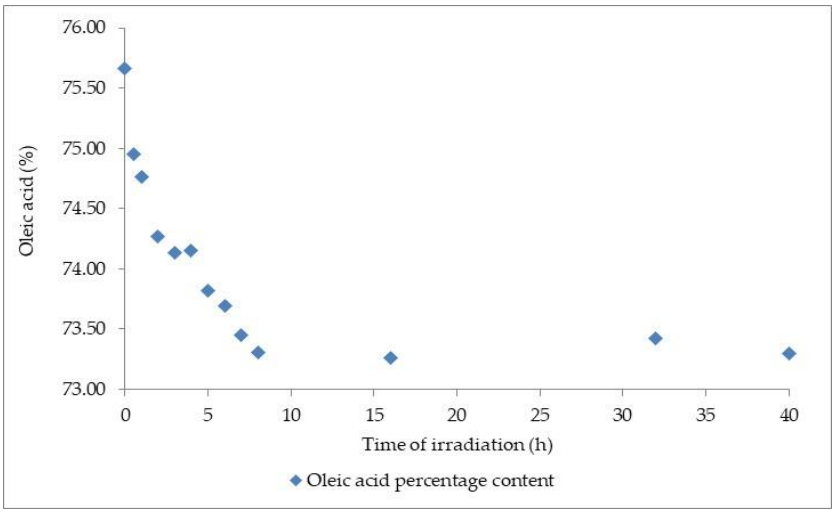

Figure 3. Percentage content of oleic acid in Cerasuola "green" (CV); sample irradiated at different time.

The higher percentage variation was detected during 8 h of irradiation, while, after 16 h, no significant percentage variation was detected.

Accordingly, the photo-oxidative experiments were carried out by irradiating the different EVOOs and mixture samples for 32 h in the same experimental conditions. The main results are showed in Figure 4.

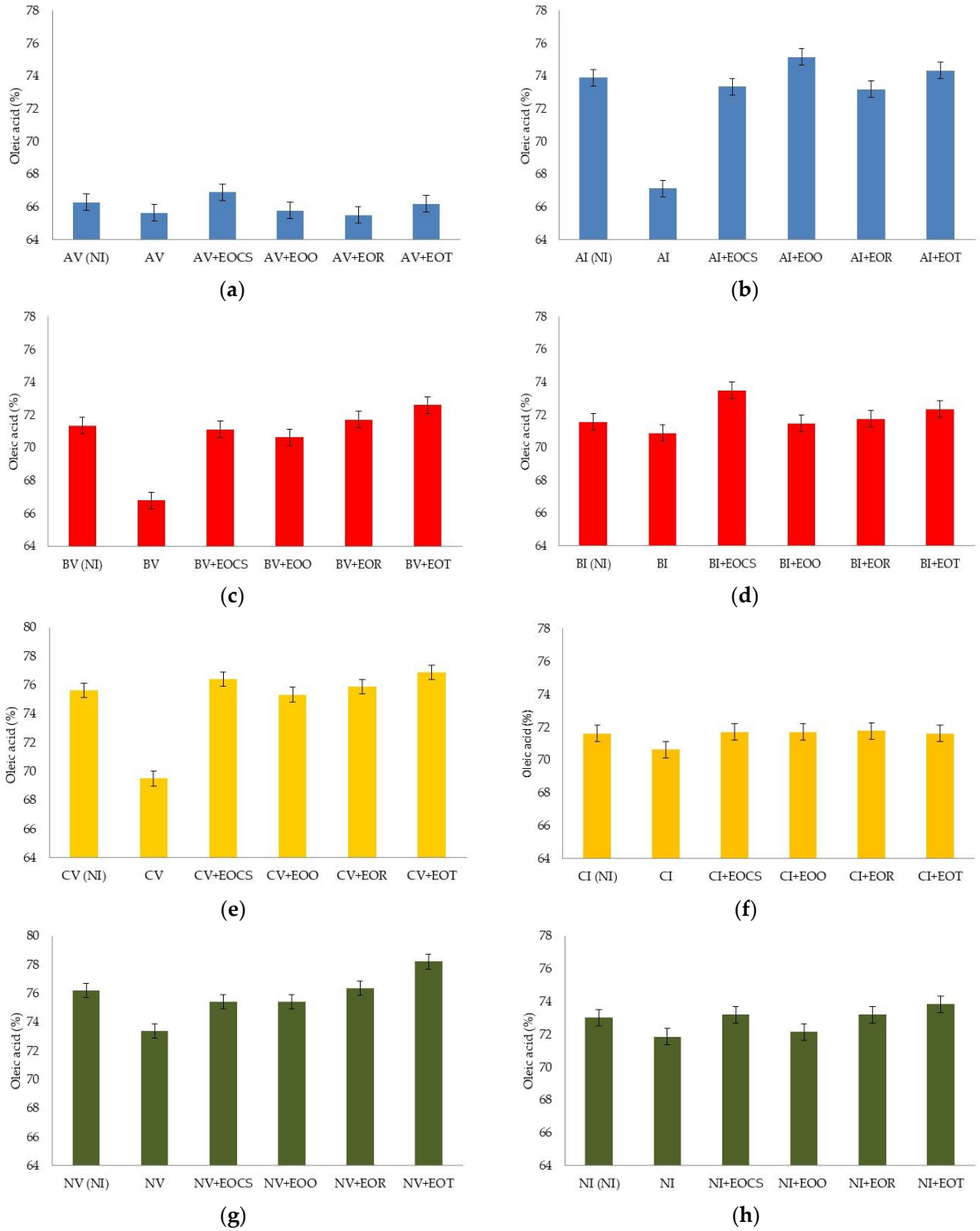

Figure 4. Percentage content of oleic acid in the extra-virgin olive oil (EVOO) samples not irradiated, irradiated and in the mixture of EVOO with EO. Average values (±standard error) are shown. The initials NI in brackets in all graphs stand for not irradiated; EOCS stands for essential oil of common sage; EOO stands for essential oil of oregano; EOR stands for essential oil of rosemary; EOT stands for essential oil of thyme. (**a**) refers to Arbequina "green" (AV), (**b**) refers to Arbequina "brown" (AI), (**c**) refers to Biancolilla "green" (BV), (**d**) refers to Biancolilla "brown" (BI), (**e**) refers to Cerasuola "green" (CV), (**f**) refers to Cerasuola "brown" (CI), (**g**) refers to Nocellara del Belice "green" (NV) and (**h**) refers to Nocellara del Belice "brown" (NI).

In all EVOOs samples exposed at irradiation performed at 360 nm, variations in oleic acid composition were detected. Furthermore, in three different EVOOs samples

(CV, BV and AI), a relevant variation in the percentage content of oleic acid (8.00%) was recorded. This variation can be ascribed at photo-oxidative reaction. In fact, as reported in literature [38], the fatty acid alkyl chain is susceptible to oxidation both at double bonds and adjacent allylic carbons. In fact, free-radical and photo-oxidative reactions at allylic carbons are responsible for deterioration of unsaturated oils and fats, resulting in rancid flavours and reduced nutritional quality. In this context, light and oxygen, promotes oxidation of unsaturated fatty acids. Ultraviolet radiation decomposes existing hydroperoxides, peroxides and carbonyl and other oxygen-containing compounds, producing radicals that initiate autoxidation. Moreover, naturally present pigments such as chlorophyll, hematoporphyrins and riboflavin act as sensitizers. Light excites these sensitizers to the triplet state that promotes oxidation processes.

No significant change of oleic acid percentage was detected in the mixture of EVOO with EO samples. These results seem to bring out the presence of an antioxidant effect of EOs on EVOO. This result is independent from the presence of phenolic compounds, such as in the oregano and thyme EOs, which have a high percentage of phenolic compounds. It would seem, therefore, a synergistic effect of all the components of EOs. Further studies are needed to highlight a correlation between the chemical composition of the MAPs EOs and antioxidant effects.

4. Conclusions

This work is an innovative study to assess the quality of flavoured EVOO prepared by adding MAPs EO. The proposed preparation method is valid to obtain flavoured EVOO and can represent a useful alternative to traditional methods. The addition of EO allows, in fact, to maintain the biological properties of EVOO and prevent any oxidation process which can occur due to light and oxygen. Furthermore, being that EO is rich in antioxidants, its addition causes an improvement of the shelf life of EVOO. The study carried out through the GC analysis can be also a support to the sensory analysis for the evaluation of the oil quality. From a health point of view, it is possible to sustain that the use of flavoured EVOO by adding MAPs EO can have several benefits for humans such as to come from natural resources, to be considered safe and to have functional and sensory properties. Furthermore, the addition of natural antioxidants from EOs, instead of synthetic ones, could be of high interest to food industry whose primary objective is to offer good, healthy and safe food products, with a balanced nutritional profile and economically accessible to all consumers. The creation of natural mixtures and "new" condiments requires, however, an evaluation of the production and availability of MAPs EOs. A high production of EO in relation to plant dry weight seems to be considered crucial in the cultivation of MAPs. Further studies are required to confirm these findings and assess the oxidative stability of the mixtures in the long term, being that the literature in this field is very minimal.

Author Contributions: Conceptualization, S.LB., A.M., A.P. and T.T.; methodology, S.B. (Salvatore Barreca), S.L.B., A.M., A.P. and T.T.; software, S.B. (Salvatore Barreca) and M.L.; validation, S.B. (Silvestre Buscemi) and C.L.; formal analysis, A.M. and T.T.; investigation, S.B. (Salvatore Barreca) and S.L.B.; resources, S.LB., A.P. and T.T.; data curation, S.B. (Salvatore Barreca) and M.L.; writing—original draft preparation, S.L.B., A.M. and M.L.; writing—review and editing, S.L.B., A.M., M.L. and A.P.; visualization, S.B. (Silvestre Buscemi) and T.T.; supervision, S.B. (Silvestre Buscemi) and C.L.; project administration, C.L.; funding acquisition, C.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Union, Italian Ministry of Education, University and Research and Italian Ministry of Economic Development, grant number: PON02_00667—PON02_00451_3361785.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available by contacting the authors.

Acknowledgments: The authors would like to thank the European Union, Italian Ministry of Education, University and Research and Italian Ministry of Economic Development, funding the "DI.ME.SA.—Valorisation of typical products of the Mediterranean diet and their use for health and nutraceutical purposes", research project.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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