Hindawi
Journal of Food Quality
Volume 2023, Article ID 6628839, 11 pages
https://doi.org/10.1155/2023/6628839

Research Article

Effect of Adding *Thymus leptobotrys* on Extra Virgin Olive Oil's Quality and Physicochemical Parameters

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Received 15 March 2023; Revised 11 April 2023; Accepted 12 May 2023; Published 2 June 2023

Academic Editor: Francisca Hernández

The present study aimed to evaluate the changes in extra virgin olive oil (EVOO) after enrichment treatments with *Thymus leptobotrys*, a Moroccan endemic aromatic plant. The EVOO sample was supplemented with two materials obtained from the aerial parts of *T. leptobotrys*: powder (2.5 and 5%) or essential oil (100 and 200 ppm). The chemical composition analysis of *T. leptobotrys* extracts (essential oil and powder methanolic extract) allowed the determination of its phenolic profile. Quality parameters (acidity, peroxide value, and specific extinction coefficients), color, fatty acids composition, minor components (pigments and phenol), and Rancimat oxidative stability of the control and enriched oils were measured. Rosmarinic acid and thymusin were the main identified compounds in the powder; thymusin was identified for the first time in the essential oil where it was predominant. Generally, the enrichments did not downgrade the oil from its initial category (EVOO), according to quality indexes. Adding *T. leptobotrys* to EVOO, especially the essential oil, enriched its phenolic profile by allowing the migration of thymusin, a lipophile flavone, without affecting its quality parameters or color.

1. Introduction

Olive oil, obtained from fresh and healthy fruits (*Olea europaea* L.) using mechanical processes without any chemical additions and refining processes, is an important source of fat in our diets and has gained significant interest in worldwide cuisine. The increasing trend in the consumption of olive oil is due to its nutritional and health benefits, mainly attributed to its high levels of monounsaturated fatty acids and a fraction of minor components such as chlorophyll, α-tocopherol, phenols, aliphatic and triterpenic alcohols, volatile compounds, and sterols. Olive oil is also a natural source of several antioxidants [1, 2]. The quality and stability of olive oil depend on the composition and quantity of this minor fraction. The International Olive Council (IOC) classifies olive oil into various categories depending on its chemical and sensory characteristics [3], with the superior category being extra virgin olive oil (EVOO). The high nutritional value and oxidative stability of this category are mainly due to its minor fraction, particularly the phenolic compounds [4]. Moreover, in Europe, as stipulated in regulation No. 432/2012 [5], health claims can be attributed to the EVOO, allowing consumers to better recognize its high quality [6].

In recent years, there has been growing interest in investigating the nutritional quality and sensory properties of EVOO to diversify products and increase their commercial value. To achieve this goal and meet consumer demand, many

studies have explored the aromatization of EVOO with selected aromatic and medicinal plants, fruits, and spices [7]. However, the absence of regulations or specific standards hampers the market, making it vulnerable to fraud, such as using enrichment to mask defects initially present in the oil [7]. Several studies have demonstrated that these treatments extend olive oil shelf-life and increase its stability [8–10]. While aromatization of EVOO can have a positive effect on its physicochemical and sensory characteristics, negative consequences have also been reported [11, 12]. Thyme species have been extensively used as aromatization agents in olive oil [13–15] due to their health benefits, particularly their antioxidant activity that can help prevent several diseases such as diabetes, cardiovascular diseases, viral, and microbial infections [16]. Various studies have evaluated the effects of flavoring EVOO with thyme using different amounts and techniques such as contact, coextraction, or incorporation of phenolic compounds extract. These studies have shown that the flavored olive oils exhibited higher oxidative stability, despite some quality parameters being negatively affected such as peroxide value or free acidity [7]. To date, there has been no report on the effect of enriching EVOO with *Thymus leptobotrys* Murb., an endemic plant of southern Morocco intensively used in traditional medicine [17]. Therefore, the main objective of this study is to investigate the effect of adding *Thymus leptobotrys* Murb. powder and essential oil on EVOO quality and physicochemical parameters and the eventual phenolic compounds transfer to the oil matrix.

2. Materials and Methods

2.1. Oil Samples. The olive oil samples were donated by a cooperative located in Amizmiz, near Marrakesh. They originate from olives harvested in mid-november 2020 from a local variety, the Moroccan picholine. The olives were crushed using a two-phase decanter centrifugation permitting the separation of the oil from the wet pomace. Samples from the obtained oil have been stored in dark bottles of a minimum volume of 250 ml at 4°C until further analysis after three months.

2.2. Enrichment Material Preparation

2.2.1. Plant Material. Thymus leptobotrys, an endemic species of Morocco, was collected from Tafraout (N29°72′/W09°74′) and identified. A voucher specimen (TL076) was deposited at the Laboratory of Microbial Biotechnologies, Agrosciences and Environment, Labeled Research Unit-CNRST N 4, Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakech, Morocco. The dried plant was ground into a powder, which was then used to enrich the EVOO and prepare a methanolic extract.

2.2.2. Essential Oil Extraction. The collected aerial parts were dried in the shade and subjected to steam distillation during 3 hours in a Clevenger apparatus. The essential oil obtained was dried over anhydrous sodium sulfate, weighed, and stored at 4°C until analysis.

2.2.3. Methanolic Extract Preparation. To characterize the compounds of the thyme powder, a methanolic extract was prepared by mixing 5 g of plant powder with methanol, stirring for 15 minutes at 4°C, and then keeping the mixture overnight at the same temperature in the dark. Afterwards, the mixture was then centrifuged (10 minutes at 5000 × g) and the supernatant was collected.

2.3. Essential Oil Chemical Analysis. The essential oil chemical profiles were determined by gas chromatography/mass spectrometry (GC/MS) method on a TRACE type 1300 model. A capillary column (TG-5MS: $L = 30$ m; $\Phi = 0.25$ mm; $Ft = 0.25\ \mu$m) was used and was programmed at 60°C for 2 min and then from 60°C to 320°C at 20°C/min. Helium was used as a carrier gas at a flow of 1 mL/min. The injection volume of essential oil samples, diluted with ethyl acetate, was 1.0 μL in split mode (1 : 50). The temperatures of the injector and detector were fixed at 280°C and 250°C, respectively. GC was interfaced with an MS (ISQ LT model) in respect of the following parameters: a source temperature and transfer line were fixed at 200°C and 250°C, operating at 70 eV and from 30 to 650 amu. Results were treated with the Xcalibur program. Comparison of component mass spectra with the mass spectra of standards and with literature data permitted the identification of individual components.

2.4. Thyme Total Phenol Content (TPC). TPC of thyme methanolic extract was determined by the Folin–Ciocalteu method [18]. 250 μL Folin–Ciocalteu reagent diluted 3 fold were added to the extract (10 μL) mixed with distilled water (1.745 mL). After 3 min, 500 μL Na_2CO_3 solution (20%) was added to the reactive mixture then incubated for 30 min at 40°C. The absorbance was measured at 760 nm. Results were expressed as gallic acid equivalents/g of dry weight (mg GAE/g DW). We utilized a calibration curve of gallic acid in methanol with concentrations ranging from 0.03 to 1 mg/ml.

2.5. Thyme Phenolic Compounds Identification. Phenolic compounds of thyme essential oil and methanolic extract, prepared as described in 2.2.3., were identified by UHLPC-DAD-ESI/MS method according to Zefzoufi et al. [19]. A Kinetex C18 reversed phase column (250 × 4.6 mm, 2.6 μm particles, Thermo Fisher Scientific, CA, USA) was used for the separation method. For methanolic extract, the mobile phase contains 0.1% formic acid aqueous solution as solvent A and methanol as solvent B, the flow rate was 1 mL/min and the multistep gradient was used as follows: 0–3 min, linear gradient from 5 to 25% B; 3–6 min, at 25% B; 6–9 min, from 25 to 37% B; 9–13 min, at 37% B; 13–18 min, from 37 to 54% B; 18–22 min, at 54% B; 22–26 min, from 54 to 95% B; 26–29 min, at 95% B; 29–29.15 min, back to initial conditions at 5% B; and from 29.15 to 36 min, at 5% B. Concerning essential oil, we used 0.1% formic acid/water as solvent A and acetonitrile as solvent D. The multistep gradient used was 0–2 min 2% D 2–20 min, 2–25% D; 20–25 min 25–35% D; 25–28 min 35–95% D; 28–30 min 95–95% with a final plateau of 2 min at 2% D; and flow rate 1 mL/min and oven

temperature 25°C. The injection volume was 10 μL. The UV–vis spectra were recorded in the range 220–800 nm and chromatograms were also acquired to 350 nm.

In this study, the chromatograms were set at 280 nm and the UV-Vis detection was accumulated in the 200–400 nm range. The following detection parameters were used for MS: a sheath gas, ion sweep gas, and auxiliary gas at flow rates of 65, 0, and 40 arbitrary units (a.u.), respectively. The ion transfer tube and vaporizer were set at temperatures of 350°C and 2500 V in negative mode with 50 V for fragmentation energy. Full scan MS acquisition mode was recorded in the range of 50–1000 m/z.

2.6. Preparation of Enriched Extra Virgin Olive Oils. Two thyme enrichment materials, powder and essential oil, were applied. Thyme powder was directly added to the EVOO at 2.5% and 5% (w/w). After 1 h of stirring, samples were maintained at 24 h of maceration, and then filtered. On the other hand, the essential oil was added separately to the EVOO at 100 and 200 ppm (w/w), and the mixtures were stirred for 1 h.

The enriched extra virgin olive oil samples were prepared in quadruplicate and stored at 4°C in dark bottles until further analysis.

2.7. Oil Physicochemical Quality Parameters. Free acidity (FA), peroxide value (PV), and specific extinction coefficients (K_{270} and K_{232}) were measured according to international standards (ISO 660; ISO 3960; ISO 3656; respectively) [20–22]. Free acidity is expressed in % of oleic acid and peroxide value is expressed as meq $O_2 \cdot kg^{-1}$.

Pheophytin and chlorophyll ($mg \cdot kg^{-1}$ of oil) content was measured at 630, 670, and 710 nm using a visible UV spectrophotometer [23, 24].

The color of oils was determined using a Lovibond PFX195 tintometer and expressed in CIE-Lab coordinates L^* (light/dark), a^* (red/green), and b^* (yellow/blue) that can fluctuate from −100 to +100 for a^* and b^* and 0 to +100 for L^*.

2.8. Oil Total Phenol Content (TPC) and Their Identification. As described by Vázquez-Roncero et al. [25], 10 g of olive oil was dissolved in hexane and extracted three times with 10 mL methanol/water (60 : 40 v/v) by shaking for 2 min. Then, 5 mL of distilled water and 1 mL of Folin–Ciocalteu reagent were added to 0.1 mL of hydro-methanolic extract solution. After 5 min, 1 mL of Na_2CO_3 solution (20%) was added to the reactive mixture. After 60 min of incubation, the absorbance was measured at 725 nm, and the total phenols were expressed as gallic acid equivalents/g of dry weight (mg GAE/g DW) using the calibration curve described in Section 2.4.

Phenolic compounds identification was performed using UHPLC-DAD-ESI/MS following the method described in Section 2.5.

2.9. Fatty Acids Analysis. Fatty acids composition was carried out by transesterification of fatty acid according to the analytical methods described in EEC Regulation 2568/91 [26]. The chromatography/mass spectrometry (GC/MS) method was used with the same apparatus described in Section 2.3. GC was programmed at 130°C for 2 min, then from 130°C to 180°C at 10°C/min, from 180°C to 245°C at 3°C/min, and from 245°C to 300°C at 15°C/min. The injector temperature was 250°C, and the carrier gas was helium. GC was interfaced with an MS (ISQ LT model) with the following parameters: a source temperature and transfer line fixed at 230°C and 250°C, respectively, operating at 70 eV and from 100 to 400 amu. Results were treated with the Xcalibur program.

2.10. Determination of Oxidative Stability. Oxidative stability was evaluated by the Rancimat. The Rancimat induction time (IT) was determined using a Metrohm Rancimat 743 (Metrom, Base, Switzerland) with a 3 g test sample of oil at an airflow of 20 $L \cdot h^{-1}$ and 100°C. Results were expressed as the oxidation induction time (in hour).

2.11. Statistical Treatment. The experimentally assessed analysis parameters were expressed as the mean ± standard deviation (SD). Differences were significant at $P < 0.05$. The analysis of the variance between the averages was performed by the one-way ANOVA test followed by student-Newman-Keuls Test. The two tests were performed using the SPSS program.

3. Results and Discussion

3.1. GC-MS Essential Oil Composition. Qualitative analytical results are shown in Table 1. GC-MS permitted the identification of eighteen components that represent 95% of the total oil compounds. Based on their chemical structures, the identified compounds were classified into four groups: monoterpene hydrocarbons (7.30%), oxygenated monoterpenes (78.42%), sesquiterpenes hydrocarbons (8.02%), and oxygenated sesquiterpenes (1.18%). The most prevalent compound was carvacrol (74.63%), followed by p-cymene (5.33%) and (E)-caryophyllene (5.05%) with thymol being a minor compound (0.19%). These results are consistent with the previous works for the same species of thyme [27, 28].

3.2. Thyme Extract TPC and UHPLC-MS Phenolic Profile. Total phenol content of the thyme methanolic extract was 11.22 ± 0.1 mg $GAE \cdot g^{-1}$dry matter which is below the results previously reported by Sayout et al. [29].

We analyzed the methanolic extract and the essential oil of *Thymus leptobotrytis* by UHPLC/DAD/ESI-MS. Peaks were identified based on their UV characteristics and on the comparison of their mass spectra (M-H) with literature reports [30–32] (Figure 1, Tables 2 and 3). Five

Table 1: Chemical composition of *T. leptobotrys* essential oil.

Compounds	KI*	Relative abundance (%)
α-pinene	933	0.90
Camphene	952	—**
Myrcene	991	0.62
α-phellandrene	1005	—
α-terpinene	1018	0.45
p-cymene	1026	5.33
γ-terpinene	1059	—
Linalool	1098	0.71
Borneol	1165	0.76
Terpinen-4-ol	1177	0.49
Carvacrol methyl ether	1244	1.64
Thymol	1290	0.19
Carvacrol	1298	74.63
(E)-caryophyllene	1428	5.05
Aromadendrene	1439	2.31
Alloaromoadendene	1461	0.66
Caryophyllene oxide	1573	1.18
Monoterpenes hydrocarbons		7.30
Oxygenated monoterpenes		78.42
Sesquiterpenes hydrocarbons		8.02
Oxygenated sesquiterpenes		1.18
Total identified (%)		94.92

Note: KI*: Kováts retention index. —**: trace.

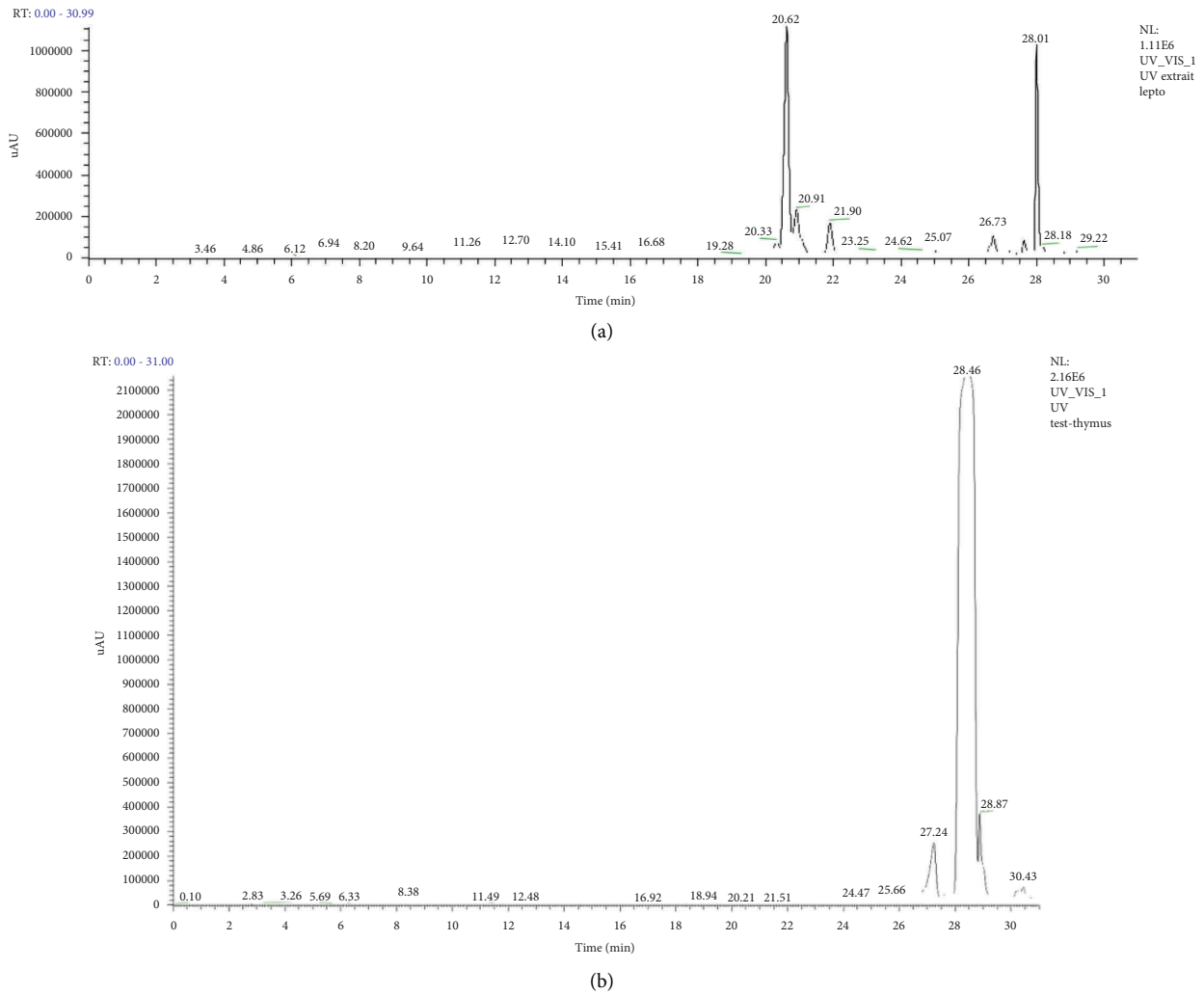

Figure 1: HPLC chromatogram of *T. leptpbotrys*: methanolic extract (a) and essential oil (b).

Table 2: UHPLC/DAD/ESI-MS of *T. leptobpotrytis* methanolic extract.

Peak no	RT (min)	UHPLC/UV (nm)	m/z (M-H)-	MS^2	Proposed compound	ME* (%)
1	14.1	275.329	593	269	Apigenin 6,8-di-C-glucoside	2.17
2	20.62	330.360	359.04	196	Rosmarinic acid	45.12
3	20.91	325	609.1	301	Rutin	1.54
4	26.73	285.343	284.9	—	Luteolin	3.12
5	28.02	210.276	329.12	—	Thymusin	41.25
			TPC (mg GAE·g^{-1} dry matter)			11.22 ± 0.1

Note: *: Methanolic extract.

Table 3: UHPLC/DAD/ESI-MS of *T. leptobpotrytis* essential oil (EO).

No	RT	UHPLC/UV (nm)	m/z (M-H)-	Proposed compound	EO (%)
1	8.2	214, 276	133.1	p-cymene	2.50
2	21.11	276	148.9	Carvacrol	3.78
3	25.29	210, 276	329.1	Thymusin	84.21
4	28.04	280	297.1	5,4′-dihydroxy-6,7-methylenedioxyisoflavone	4.34
5	31.14	280	339.1	Unknown	1.17

Table 4: Physicochemical quality parameters, TPC, fatty acids composition, and oxidative stability of control olive oil (EVOO), enriched olive oil with powder (VOOP), and essential oil (VOOEO) of *T. leptobotrytis*.

Parameter	IOC fixed limits for EVOO	EVOO	VOOP		VOOEO	
			2.5%	5%	100 ppm	200 ppm
FA (%)	≤0.8	0.2431 ± 0.0003^{d}	0.481 ± 0.005^{a}	0.483 ± 0.003^{a}	0.294 ± 0.066^{c}	0.3540 ± 0.0053^{b}
PI (meq O_2·kg^{-1})	≤20	14.78 ± 1.85^{ab}	15.88 ± 1.80^{ab}	17.15 ± 1.83^{a}	10.48 ± 0.96^{c}	12.49 ± 2.49^{bc}
K232	≤2.5	1.83 ± 0.04^{a}	1.69 ± 0.07^{a}	1.64 ± 0.13^{a}	1.69 ± 0.17^{a}	1.6 ± 0.07^{a}
K270	≤0.22	0.21 ± 0.01^{c}	0.26 ± 0.01^{b}	0.29 ± 0.02^{a}	0.22 ± 0.01^{c}	0.23 ± 0.01^{c}
Chlorophyll (ppm)		1.19 ± 0.01^{c}	2.82 ± 0.10^{b}	4.37 ± 0.38^{a}	1.16 ± 0.01^{c}	1.37 ± 0.15^{c}
Phepphytin (ppm)		3.95 ± 0.03^{c}	9.37 ± 0.03^{b}	14.55 ± 1.32^{a}	3.873 ± 0.26^{c}	4.56 ± 0.52^{c}
L^*		65.96 ± 2.54^{a}	40.21 ± 0.52^{b}	40.60 ± 0.48^{b}	66.91 ± 3.59^{a}	64.30 ± 3.52^{a}
a^*		-6.20 ± 0.21^{c}	-3.7 ± 0.20^{b}	-1.75 ± 0.04^{a}	-6.74 ± 0.38^{c}	-6.47 ± 0.95^{c}
b^*		52.0 ± 2.0^{b}	51.0 ± 1.0^{a}	59.0 ± 0.5^{b}	51.4 ± 2.4^{b}	50.2 ± 1.2^{b}
TPC (mg GAE·g^{-1} dry matter)		159.66 ± 22.87^{a}	179.98 ± 16.76^{a}	151.94 ± 34.18^{a}	160.46 ± 27.71^{a}	155.72 ± 17.61^{a}
IT (h)		34.45 ± 0.11^{ab}	34.35 ± 0.60^{ab}	34.06 ± 0.62^{b}	35.12 ± 0.21^{a}	35.32 ± 0.66^{a}

compounds were identified in extracts and classified into three subgroups of phenolic compounds: phenolic acids (rosmarinic acid), flavone (thymusin and luteolin), and flavonol glycoside (apigenin 6,8-di-C-glucoside and rutin). The methanolic extract is characterized by the predominance of rosmarinic acid and thymusin (Table 2). Rubió et al. [13] had already revealed the predominance of rosmarinic acid and thymusin in *Thymus zygis* extract. However, *Thymus vulgaris* was lower in rosmarinic acid [33]. Thymusin was also detected in *Thymus fontansii* leaves extract [31].

Concerning essential oil, the chromatographic profile (Table 3) revealed five compounds subdivided into two families: terpenoids and flavonoids. For terpenoids, the presence of oxygenated terpenoids (carvacrol, m/z = 148.9) and terpenes hydrocarbons (p-cymene, m/z = 133.1) is noticed. Regarding flavonoids, thymusin (84.21%) is the most abundant component, followed by 5,4′-Dihydroxy-6,7-methylenedioxyisoflavone (4.34%). As per our research, up to now, there is no report on the presence of thymusin in essential oil from *T. leptobotrys*.

3.3. Effect of Enrichment on Oil Quality Parameters. Different effects of olive oil enrichment on its quality parameters were reported which might be explained by differences in the added agent composition and the nature of their bioactive components, flavoring processes, concentration level, and olive oil characteristics [14]. Cultivars [34] and period of storage is also a determinant factor [35, 36].

Results for quality parameters are summarized in Table 4. Free acidity values are significantly higher for all the enriched oils than for control. Additionally, powder increased free acidity more than essential oil. Obtained values, ranging from 0.24 to 0.48%, were below the limit fixed by the IOC for the EVOO category suggesting that aromatization did not down ground the oil from its initial category. The presence of organic acids in thyme, causing a more acidic environment that probably allowed the hydrolysis of triglycerides, could explain the increased acidity level in enriched oils [37].

Concerning the peroxide values, oil enriched with essential oil at 100 ppm is significantly lower than the control and might provide initial evidence of the contribution of thyme in

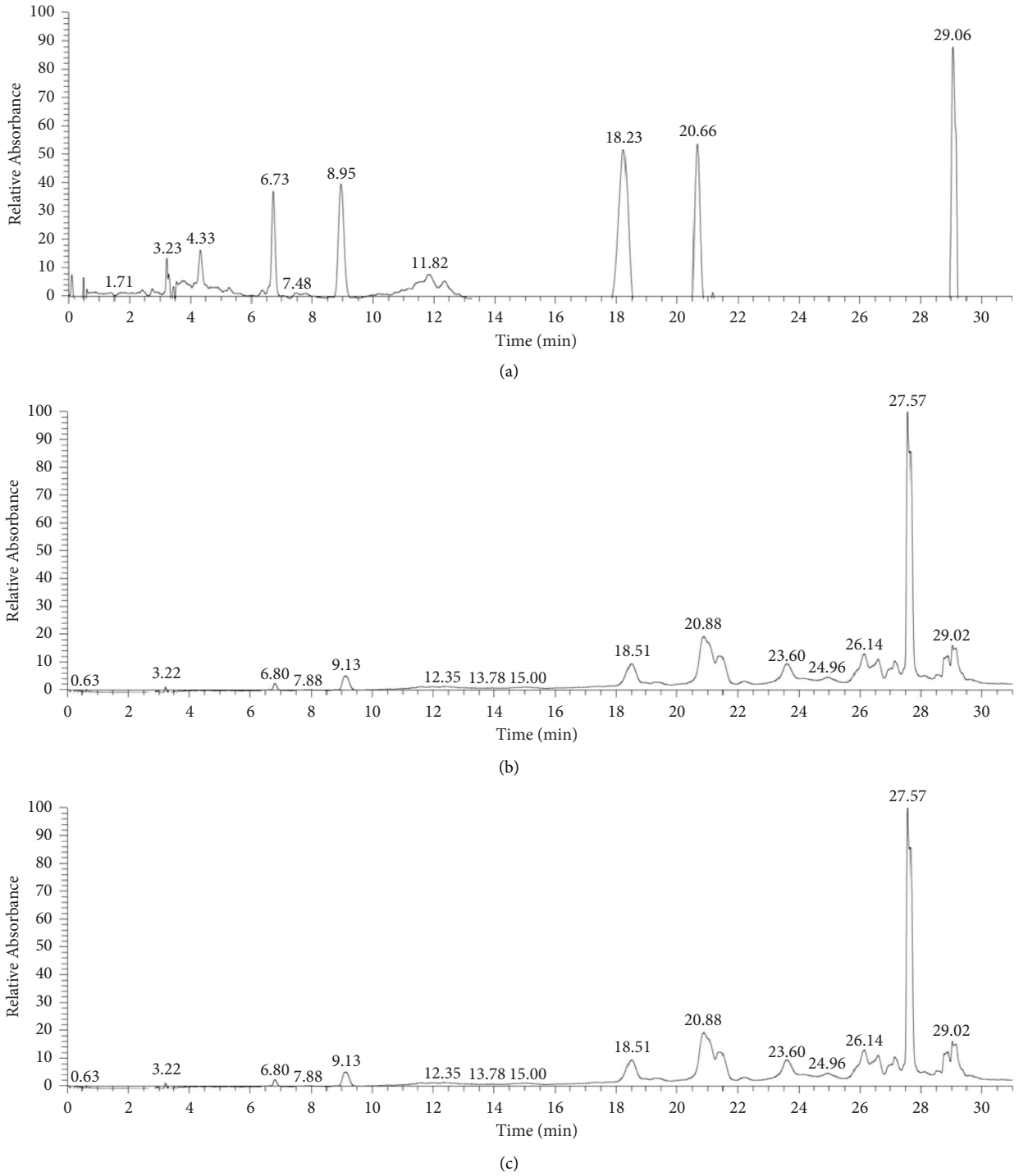

Figure 2: HPLC chromatogram of control olive oil (a), enriched oil with powder (b), and with essential oil (c) of *T. leptobotrytis*.

preserving the antioxidant activity. However, enrichment by powder at 5% raised the PV which is in agreement with the results on other aromatization works [35, 38]. Regardless of the variations observed (10.48 to 17.15 meq $O_2 \cdot kg^{-1}$), all oils remained in the extra virgin category. Other works revealed either an increase or a decrease depending on the type of enrichment, as was reported for enrichment with Brazilian pink pepper and thyme, respectively [12, 39].

No significant enrichment effect was noticed for K_{232} values (1.64 to 1.83) that were under the IOC fixed limit for EVOO. However, enrichment with powder increased K_{270} (0.21 to 0.29), exceeding the IOC set limit for EVOO. The passage of the components from the plant to the oil absorbing at 270 nm or the apparition of secondary oxidation products could explain this result. In the previous works, the extinction coefficients were sometimes higher [12, 37]

Table 5: UHPLC/DAD/ESI-MS of control olive oil and enriched with powder and essential oil of *T. leptobotrytis*.

N°	RT	HPLC/ UV	m/z (M-H)-	Fragments	Proposed compound	EVOO (%*)	VOOP (%*)		VOOEO (%*)	
							2.5%	5%	100 ppm	200 ppm
1	7.05	210, 280	153.20	111/97.18/79.2	Hydroxy-tyrosol	4.780 ± 0.126	0.120 ± 0.002	0.1000 ± 0.001	3.77 ± 0.226	3.82 ± 0.091
2	9.53	280	318.91	217/177.04	Decarboxymethyl oleuropein aglycone	8.61 ± 0.13	5.17 ± 0.09	4.08 ± 0.27	6.85 ± 0.19	6.90 ± 0.11
3	18.97	280	318.95	217/177.03	Decarboxymethyl oleuropein aglycone isomer	17.59 ± 0.15	10.26 ± 0.49	6.90 ± 0.35	14.85 ± 0.31	13.54 ± 0.04
4	21.24	222, 276	377	291/259	Oleuropeine aglycone	22.45 ± 0.44	15.96 ± 0.05	8.80 ± 0.04	18.91 ± 0.48	17.68 ± 0.08
5	21.8	220, 276	361.15	291/259/217/ 177	Ligstroside aglycone	4.42 ± 0.34	3.13 ± 0.05	3.57 ± 0.14	4.34 ± 0.44	3.99 ± 0.37
6	24.2	220, 276	319.08	307/275/217.13	Oxidized product of aldehydic form of ligstroside aglycone	6.58 ± 0.17	5.41 ± 0.04	4.55 ± 0.14	5.53 ± 0.17	4.85 ± 0.14
7	25.62	220, 276	319.03	307/275/217	Oxidized product of aldehydic form of ligstroside aglycone isomer	5.60 ± 0.24	3.80 ± 0.14	2.96 ± 0.12	4.84 ± 0.22	4.07 ± 0.01
8	26.45	280	285.17	—	Luteolin	13.12 ± 0.39	11.63 ± 0.07	9.94 ± 0.34	12.00 ± 0.39	11.43 ± 0.06
9	26.90	220, 276	318.91	307/275/217	Oxidized product of aldehydic form of ligstroside aglycone isomer	6.63 ± 0.19	5.00 ± 0.04	4.30 ± 0.03	5.59 ± 0.19	5.28 ± 0.01
10	27.27	236, 336	269.16	—	Apigenin	4.83 ± 0.11	2.11 ± 0.06	1.63 ± 0.12	3.04 ± 0.11	2.43 ± 0.03
11	27.65	244	329.38	311/291	Thymusin	—	32.13 ± 0.04	47.88 ± 0.80	14.89 ± 3.24	20.60 ± 0.69

Note: *: Peak area %.

Table 6: Fatty acids composition of control olive oil and enriched with powder and essential oil of *T. leptobotrytis*.

Fatty acid	EVOO (%*)	VOOP (%*)		VOOEO (%*)		IOC value
		2.5%	5%	100 ppm	200 ppm	
C16: 0	11.99	11.89	11.74	12.11	13	7.5–20.0
C16: 1	0.75	0.69	0.71	0.73	0.77	0.3–3.5
C17: 0	0.05	0.06	0.05	0.06	0.05	<0.3
C17: 1	0.03	0.03	0.03	0.03	0.03	<0.6
C18: 0	2.22	2.08	2.09	2.13	2.29	0.5–5.0
C18: 1	65.41	66.5	66.46	65.82	64.58	55.0–83
C18: 2	16.6	15.89	15.95	16.1	16.71	3.5–21.0
C18: 3	0.85	0.91	0.99	0.87	0.77	0.0–1.5
C20: 0	0.03	0.02	0.02	0.02	0.02	<0.6
C20: 1	0.22	0.18	0.18	0.17	0.18	<0.4
SFA	14.29	14.05	13.9	14.32	15.36	
UFA	83.86	84.2	84.32	83.72	83.04	
UFA/SFA	5.87	5.99	6.07	5.85	5.41	
MUFA/PUFA	3.99	4.24	4.23	4.14	3.92	

Note: *: Peak area (%). Saturated fatty acid (SFA), unsaturated fatty acid (UFA), mono unsaturated fatty acid (MUFA), and poly-unsaturated fatty acid (UFA).

and other times lower [39] in enriched oils than that in control.

Pheophytin and chlorophyll values ranged respectively from 3.95 to 14.56 mg·kg^{-1} and from 1.19 to 4.37 mg·kg^{-1}. These parameters were higher in oils enriched with powder which could be due to the passage of pigments from the powder to the oil and might then explain the higher PV obtained with powder at 5%. This result could be concordant with the pro-oxidant nature of chlorophyll. However, chlorophyll can also act as an antioxidant. A high amount of it could be considered in enhancing oxidative stability, which is probably the case for the other enrichments and as has been reported in other works [40].

Moreover, all oils revealed a negative value of the chromatic ordinate a^*, classifying them in the green zone, a positive value of chromatic ordinate b^* corresponding to the yellow zone, while L^* values ranged from 40.21 to 66.91. Oils enriched with powder showed a decrease for L^* and a dose-dependent increase for the coordinate a^*, which can mean a loss of clarity and a gain in color intensity related to the observed pigments concentration. These results could be explained by the relationship between the values of chromatic ordinates and the evolution of pigments, as observed in a precedent study [41].

3.4. Effect of Enrichment on Oil TPC and Phenolic Profile. Olive oil enrichment with the used thyme materials did not show a significant difference in total phenol content levels varying from 151.94 ± 34.18 to 179.98 ± 16.76 mg GAE·g^{-1} dry matter (Table 4). In a similar way, Fagundes et al. [12] did not note any difference in TPC between flavored treatments by Brazilian pink pepper but obtained a slight increase compared to the control group. In other enrichments with Thymus, different trends of TPC level variations were reported [13, 14].

To identify the phenolic compounds in the EVOO and the enriched VOO with *T. leptobotrytis*, the UHPLC/DAD/ESI-MS was performed. Eleven compounds were identified on the basis of the UV spectrum and mass spectra compared with the literature [31, 42, 43] (Figure 2, Table 5). All samples showed a high diversity of secondary metabolites divided into terpenoids and phenolic compounds. Secoiridoids are the main terpenoids, especially secoiridoids aglycone (oleuropeine aglycone, decarboxymethyl oleuropein aglycone and its isomer, and ligstroside aglycone) and secoiridoids aldehyde form. Secoirodoids are reported to be the main group of phenolic compounds in olive oil [44, 45]. Two subgroups of phenolic compounds were also observed in all samples: phenylethanoid (hydroxy-tyrosol) and flavone (luteolin a d apigenin). In the enriched VOO, another flavone was detected and identified as thymusin. Indeed, as per our results, thymusin is a predominant compound in essential oil and total methanolic extracts of *T. leptobotrytis* (Tables 2 and 3). The presence of thymusin in the enriched VOO could be explained by its passage from thyme to the enriched oils. The obtained oil would be richer in flavonoids that are known to offer health benefits (neuroinflammation decrease and attenuation of oxidative stress). Thymusin has been already detected in oil flavored with Thymus zygis extract [13] but up to now, there is no report on the presence of thymusin in *T. leptobotrys*. Some phenols abundant in thyme and not detected in the enriched VOO, such as rosmarinic acid, would not have been soluble in oil.

3.5. Effect of Enrichment on Oil Oxidation Stability. Oxidative stability is a powerful indicator of edible oil quality. It was measured, for all oils samples, by the Rancimat test. Results (Table 4) revealed good oxidative stability for all oils. Thus, enrichment procedures did not affect enriched VOO oxidative stability. It was reported that oxidative stability depends on many factors linked to oil composition, processing methods, conservation conditions, etc., [46, 47].

3.6. Effect of Enrichment on Oil Fatty Acids Composition. Results of fatty acids composition (Table 6) comply with the IOC standard [3], and no difference between control and enriched VOO were observed. Fagundes et al. [12] reported the same trend. Ten compounds were identified for each oil,

and the main fatty acid was oleic acid (C18: 1), followed by linoleic acid (C18: 2), palmitic acid (C16: 0), and stearic acid (C18: 0) when in fact palmitoleic, linolenic, arachidic, gadoleic, and margaroleic acids were present in minor amounts (<2%). Total monounsaturated fatty acids (MUFA), known for preventing cardiovascular diseases, were predominant and followed by poly-unsaturated (PUFA) and saturated fatty acids. The ratio MUFA/PUFA varied from 3.92 to 4.32 and confers a nutritional value to the different oils.

4. Conclusion

There has been an emergent trend of olive oil enrichment during the last few years as a response to the new consumer need for healthier products with new sensory sensation. Current standards and legislations do not allow the enriched oils to keep its extra virgin olive oil category. This work permitted the study to evaluate the effect of *T. leptobotrys* addition on the EVOO quality and physicochemical parameters that can compensate for this loss of designation.

Results revealed the richness of *T. leptobotrys* in phenolic compounds such as rosmarinic acid, luteolin, and thymusin. This last compound was detected as a main phenolic compound in essential oil.

The results confirm that the addition of *Thymus leptobotrys* did not show a negative effect on the chemical composition of the olive oil nor on the majority of the physicochemical parameters, thus remaining within the limits set by the IOC for the extra virgin olive oil category. Furthermore, it allowed the passage of lipophilic flavonoids known to offer health benefits. The sensory aspect will be evaluated in the perspective of this study to measure its organoleptic acceptance and compliance with the IOC standards. Finally, we conclude that apart from the slight increase of K_{270}, adding thyme powder in a lower amount could be more appropriate than essential oil because it remains a simple process to develop new products and avoid secondary oxidation.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure

Aouatif Aboudia is presently at FST de Marrakech, Marrakesh, Morocco.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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