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Original Article

# Bioenrichment using *Satureja montana* L. essential oil for the prevention against photooxidation of flavored extra virgin olive oil during light display

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## Abstract

**Background:** In response to consumer demand for novel and healthy foods, the presence in the market of olive oils (OOs) flavored with different plants, spices, herbs or fruits is increasingly common. All these flavoring agents have been used over the years due to its content in compounds with biological activities. **Aim:** The aim of this study was to investigate the potential role of the addition of *S. montana* EO at 100 ppm (0.01%, v/v), known for its high content of bioactive compounds, good flavor, and aroma in improving oxidative stability and quality profile of EVOO subjected to conditions causing accelerated oxidation (Light storage at 900 lux). **Materials and methods:** The *S. montana* EO chemical components were identified using Gas Chromatography–Mass Spectrometry (GC/MS). Enriched and non-enriched EVOO samples were examined as function of time (30, 60 and 9 days) of display for different quality indices. **Results:** Using GC/MS analysis of *S. montana* EO: thymol (28.36%), carvacrol (17.45%), *p*-cymene (10.91%), trans-caryophyllene (5.54%), ɤ-terpinene (5.03%) and geraniol (4.50%) were identified. The results highlighted that the enrichment with *S. montana* EO led to lower values of lipid oxidation indicators ($K_{232}$, $K_{270}$, peroxide value) and higher concentration of antioxidants (total phenols and pigments). In sum, the use of bioenrichment methods could be a sustainable solution for the promotion of the quality characteristics of EVOO in Algeria.

**Keywords:** Bioenrichment, *Satureja montana* L., Essential oil, Extra virgin olive oil, Display, Quality stability.

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## 1. Introduction

As it has been described as functional foods, endowed with a high nutritional quality and a healthy profile, making it different from other vegetable oils, olive oil (OO) constitutes a primary source of fat and one of the main ingredients of the Mediterranean diets. Unfortunately, the unsaturated fatty acid profile makes oxidation the primary cause of deterioration of OO quality, therefore the food and olive industry are interested in finding efficient strategies for the preservation of product positive attributes [1]. The quality and the oxidative stability of OO depend on extraction technology and storage conditions (fruit storage, oxygen (O2), temperature, and packaging). Oxidation may take place either in the presence of light (photo-oxidation) or in the dark (auto-oxidation), and also catalyzed by the effect of enzymes (enzymatic oxidation). Compared with others vegetable oils, OO has a high resistance to oxidative deterioration mainly due to its monounsaturated fatty acid composition (56 to 84% of oleic acid) and its minor compounds (phenolic and orthodiphenolic compounds, carotenoids, tocopherols, and pigments) of effective antioxidant activity [2]. Recent studies have revealed an increasing interest by industry in aromatized olive oils (OOs), where the addition of essential oils (EOs) can ameliorate nutritional and organoleptic quality and, occasionally, increase the shelf-life [3]. Incorporation of some aromatic plants into OOs has improved their thermal resistance and stability against lipid oxidation [4–7].

Bioenrichment with EOs offers very promising approaches to increase the oxidative stability of foods [8-11]. Several EOs such as oregano (*Origanum vulgare*), basil (*Ocimum basilicum*), rosemary (*Rosmarinus officinalis*), laurel (*Laurus nobilis*), sage (*Salvia officinalis*), and mint (*Mentha spicata*) were found to have a positive antioxidant effect in OO [12–14]. Virgin olive oils (VOOs) are oils obtained from the fruit of the olive tree (*Olea europaea* L.), and which have not undergone any exogenous treatment. For a VOO to be considered extra, it must meet two conditions: One of a chemical nature, summarized in the percentage of acidity (≤0.8°), and another of an organoleptic nature (flavor and aroma), which is confirmed by a sensory panel. Still, the fact is that how can VOO be considered extra when substances added to it prevent sensory analysis to confirm that it is really extra virgin olive oil (EVOO) grade? Currently, the International Olive Council (IOC) has been questioned several times about OO labelled as "flavored EVOOs" mixed with extracts and EOs from various plants. One of the most aromatic plants in the Mediterranean region, *S. montana* (Lamiaceae), it's essential oil (EO) possesses potential

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interest in food industry. This EO is utilized as a flavoring agent. Algerian *Satureja* spp. Leaves have been used in food preservation (fish, figs, tomatoes, and meat) [12]. Since EVOO is the virgin oil with the highest market price and the most restrictive quality criteria established by IOC, this oil was used in the experimentation. Furthermore, since OO can rapidly oxidize when exposed to light [13,14], storage under fluorescent light was applied as accelerated degradation conditions compared to the ideal condition of darkness. The aim of this work was to investigate the potential role of the addition of *S. montana* EO, known for its high content of bioactive compounds, good flavor and aroma in improving oxidative stability and quality profile of EVOO subjected to conditions causing accelerated oxidation.

## 2. Materials and Methods

### 2.1 Materials

Dried leaves of *S. montana* was hydrodistilled using Clevenger-type apparatus and was purchased from the Florame laboratory (Florame-St Rémy de Provence- certified by Ecocert SAS F33600, France). The purchased EO has been stored only for a few months (2-3 months) after supply and stored in its original opaque sealed vial under darkness at refrigeration temperature (4 ± 1 °C) before use. *S. montana* EO was examined for its chemical profile by GC/MS analysis. The antioxidant activity was also investigated with the DPPH assay.

EVOO was obtained from Chemlal variety located in the area of M'Chedallah (Algeria) during 2014/2015, located on the southern slope of the Djurdjura mountain chain (North-Center, Algeria: 440 m (average) of altitude) at geographic coordinates Latitude 36°21'56" (North), Longitude 4°16'16" (East). The olive fruits were harvested at the optimal ripening stage (at the end of December). After harvesting, the olive fruits were immediately transported to the oil mill where they were sorted, weighed, stored, and washed. After that the olives were crushed with a millstone and presses and mixed. Then, the paste obtained was centrifuged to extract the oil. Finally, the EVOOs were decanted and immediately stored in the dark in 5L amber plastic bottles at ambient temperature (≈15 °C) until analysis. The influence of display conditions (light × darkness) and *S. montana* EO bioenrichment on EVOO stability and quality were then examined.

### 2.2 EVOO samples preparation and display conditions

This manuscript focuses on the benefits coming from the enrichment of an EVOO with an EO, taken into account that if EVOO mix with another type of oil loses its virgin character and therefore also its commercial category of extra. However, it is increasingly common to find products labelled as extra-flavored VOO in the markets.

Aiming to determine the optimal concentration of EO, to be supplemented to EVOO, a preliminary sensory analysis was carried out. Six (06) different levels of *S. montana* EO addition from 0.01 to 1% were tested (data not shown). The hedonic test was realized according to the EU Protocol (European Regulation 1348/2013) by 15 panelists.

The experimentation was carried out on February 2015. 0.01% of *S. montana* EO was added to EVOO (Obtained optimal concentration by panelists) [15]. After that, 200 mL of these EVOO were dispended into 250 mL brown glass bottles, hermetically sealed, and divided in two groups. The first group was stored in darkness and the second one was displayed under fluorescent light where bottles were exposed horizontally to a continuous fluorescent light intensity of 900 lux (measured using a luxometer 810; Chauvin Arnoux; Paris, France) and rotated every 24 h to minimize both possible abuse temperature and light intensity differences at the surface of samples). Light exposition was obtained with white fluorescent tubes (OSTRAM-L40w/19-1, Germany) placed 90 cm above the bottles. All the samples were stored at an ambient temperature (25 ± 2 °C). For each type of OO (with and without *S. montana* EO) and display conditions (dark or light), three hermetically sealed bottles were analyzed at each sampling time: 30, 60, and 90 days.

### 2.3 Chemical characterization of *S. montana* EO

The *S. montana* EO chemical components were identified using GC/MS (Agilent; model 6800 and 7973). The analysis was operated under the following conditions: capillary column (length = 30 m × 0.25 mm i.d., film thickness = 0.33 µm) coated with a HP5-MS stationary phase; ion source temperature of 230 °C. For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used over a scan range of 30–550 atomic mass units (amu); helium was the carrier gas (0.5 mL/min); set temperatures were: 250 and 280 °C for the injector and detector MS transfer line; 60 °C for 8 min, then to 280 °C at 2 °C/min and 30 min at 280 °C for the column temperature. The Kovats method was used to determine the retention indices of all constituents. The compounds were identified according to their retention indices and by comparison of their mass spectra with those of literature data [16], using the Wiley 7N, NIST 02, and NIST 98 libraries. Comparison of retention indices relative to C7-C29 n-alkanes assayed under the same conditions as EO was carried out as further confirmation of the results. The GC peak areas, calculated as the mean value of two injections, were normalized and used to calculate the composition percentage of the EO (as % of the identified compounds).

### 2.4 DPPH radical scavenging capacity of *S. montana* EO and EVOO

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH, from Alfa Aesar, Ward Hill, MA, USA) was used for the determination of the free radical-scavenging activity of the EO [17]. Different concentrations of EO were added, at an equal volume (975 µL), to an ethanolic solution of DPPH (60 mM). After 30 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated three times. Butylated hydroxytoluene (BHT, supplied by Sigma, St. Louis, MO, USA) was used as standard controls. $IC_{50}$ values denote the concentration of the sample, which is required to scavenge 50% of DPPH free radicals. For the analysis of EVOO, the measurement of DPPH was conducted using the analytical methods described by Kalantzakis *et al.* [18]. EVOO samples were diluted in ethyl acetate (10%, w/v);

1 mL was added to 4 mL of a freshly prepared DPPH solution (10-4 M in ethyl acetate) then the reaction mixture shaken vigorously for 10 s in a Vortex apparatus. After 30 min in the dark, the absorbance was measured at 517 nm against a blank solution (without radical).

The absorbance was read against pure methanol at 517 nm and the percentage of DPPH• radical scavenging activity (RSA) was calculated using the following equation:

$$RSA\% = [(Abs_{(DPPH)} - Abs_{(sample)})/ Abs_{(DPPH)}] \times 100$$

where $Abs_{(DPPH)}$ is the absorbance value at 517 nm of the ethanolic solution of DPPH and $Abs_{(sample)}$ is the absorbance value at 517 nm EVOO. Antioxidant activity based on DPPH assay was expressed as percent reduction in specific DPPH absorbance, RSA%) of bioenriched EVOO during display.

### 2.5 Application of *S. montana* EO by flavoring EVOO under conditions causing accelerated oxidation

Figure 1 shows the experimental design. The Algerian EO of *S. montana* was obtained, and batches of EVOO samples were prepared (control and treated samples) and maintained at 25 °C under accelerated shelf-life test (darkness and light conditions) during 90 days. Accelerated shelf-life test and 90 days of display has been chosen to make a practical simulation in the sale of olive oil bottles at supermarkets. The chemical composition of *S. montana* EO was determined. Displayed EVOO samples were analyzed for total phenols, total carotenoids, chlorophyll, antioxidant capacity, and quality indices ($K_{270}$, $K_{232}$, and Peroxide Value).

#### a. Physicochemical analysis of EVOO

The oxidative status of EVOO was evaluated by the measurement of Peroxide value (PV, as mEq $O_2$/kg, milliequivalents of active oxygen per kg) and extinction coefficients at 232 and 270 nm ($K_{232}$ and $K_{270}$). These physicochemical parameters were conducted following analytical protocols described in the European Union Commission Regulations [19].

#### b. Chlorophylls and carotenoids contents in EVOO

Pigments (chlorophylls and carotenoids) analysis was carried out as reported previously by Minguez-Mosquera *et al.* [20]. The chlorophyll and carotenoid fractions were measured in a spectrophotometer at 670 and 470 nm, respectively. The specific extinction coefficient (100 mL/g/cm) of 613 for pheophytin (the major component of chlorophylls) and of 2000 for lutein (the major carotenoid) were then used to calculate and express the pigments content as mg/kg.

#### c. Total phenols content in EVOO

Based on the procedure described by Gutfinger [21], total phenols content (TPC) of EVOO samples was determined with the Folin-Ciocalteu's assay. The phenolic extract of OO (0.25 mL) dissolved in ethanol was mixed with Folin-Ciocalteu reagent 10 times diluted (1.25 mL) and sodium carbonate (75 g/L, 1 mL). After incubation for 30 min at 40 °C, the absorbance at 760 nm was measured. Gallic acid (supplied by Sigma, St. Louis, MO, USA)

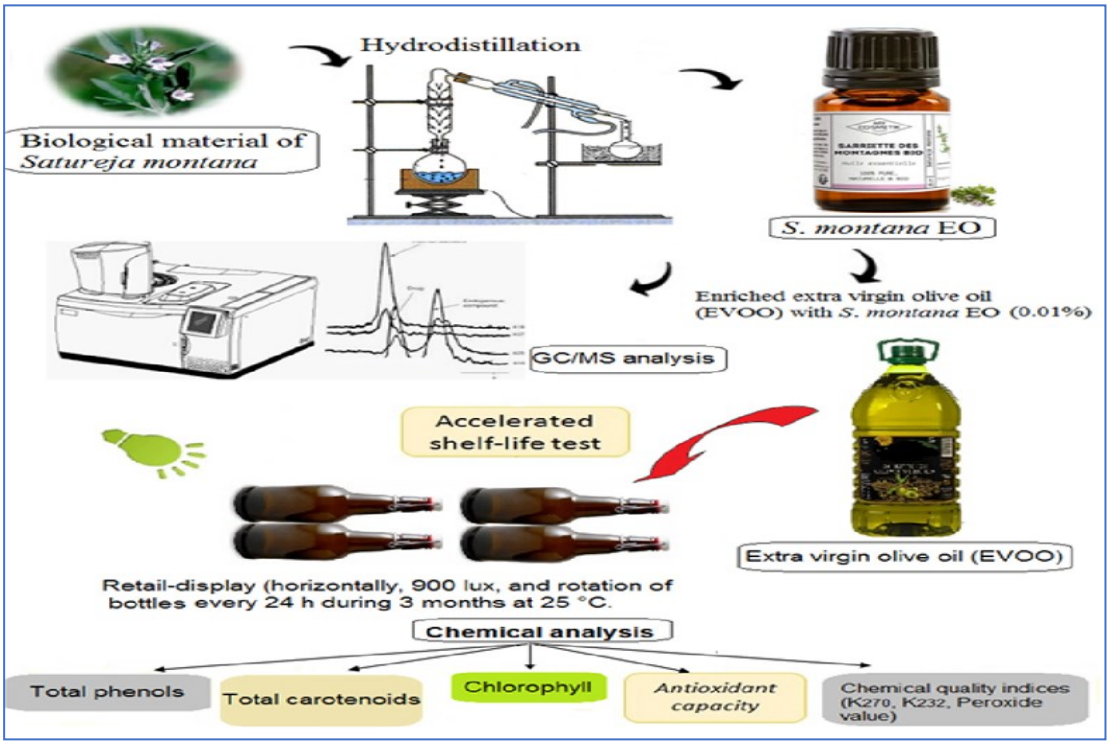


**Figure 1:** Experimental design

**Table 1:** Chemical composition (%) of *Satureja montana* L. Essential Oil (*S. montana* EO) analysed by Gas Chromatography–Mass Spectrometry (GC/MS)

| N° | Compounds | RT (min) | RI | Area (%) |
|---|---|---|---|---|
| 1 | α-Thujene | 9.72 | 921 | 0.44 |
| 2 | α-pinene | 10.10 | 926 | 0.68 |
| 3 | Camphene | 10.97 | 957 | 0.42 |
| 4 | 1-Octen-3-ol | 13.40 | 962 | 0.72 |
| 5 | β Myrcene | 13.77 | 990 | 0.81 |
| 6 | α-Terpinene | 15.45 | 1020 | 1.03 |
| 7 | *p*-Cymene | **16.32** | **1026** | **10.91** |
| 8 | β-Phellandrene | 16.46 | 1044 | 1.08 |
| 9 | β-Ocimene | 17.07 | 1052 | 0.74 |
| 10 | γ-Terpinene | 18.59 | 1057 | 5.03 |
| 11 | Cis-sabinene hydrate | 19.32 | 1058 | 0.72 |
| 12 | Linalool | 21.93 | 1104 | 2.75 |
| 13 | L-Menthone | 25.29 | 1148 | 0.47 |
| 14 | Borneol | 26.53 | 1167 | 1.95 |
| 15 | α-Terpineol | 27.23 | 1190 | 1.36 |
| 16 | Carvacrol methyl ether | 31.79 | 1263 | 1.09 |
| 17 | Pulegone | 32.29 | - | 1.99 |
| 18 | Geraniol | 33.99 | 1272 | 4.50 |
| 19 | Neral | 34.75 | 1242 | 0.47 |
| 20 | Thymol | **37.02** | **1280** | **28.36** |
| 21 | Carvacrol | **37.67** | **1297** | **17.45** |
| 22 | Geranyl acetate | 41.29 | 1440 | 0.64 |
| 23 | Trans-Caryophyllene | 43.38 | - | 5.54 |
| 24 | Aromadendrene | 44.42 | 1449 | 0.52 |
| 25 | β-Bisabolene | 48.84 | 1540 | 0.89 |
| 26 | Caryophyllene oxide | 53.17 | 1600 | 1.99 |
| | **Monoterpenes hydrocarbons** | | | **21.14** |
| | **Oxygenated monoterpenes** | | | **61.75** |
| | **Sesquiterpenes** | | | 6.95 |
| | **Oxygenated sesquiterpenes** | | | 1.99 |
| | **Others** | | | 0.72 |
| | **Total** | | | **92.55** |

RT denotes retention time; RI denotes retention index relative to n-alkanes (C7-C29) on non-polar HP5MS capillary column

served as a standard for preparing the calibration curve ranging 25-200 mg/L. The concentration of TPC is expressed as mg of gallic acid equivalents (GAE) per Kg of OO.

### 2.6 Statistical Analysis

Results have been reported as mean ± standard deviation (SD) of at least triplicate analytical determinations for each technical replicate. Influence of EO enrichment, storage time, and storage conditions (light or darkness) and their first- and second-order interactions on the evaluated parameters was assessed by three-way ANOVA using STATISTICA software version 6. Differences at $p<0.05$ were considered significant. In case of significant difference, the means were discriminated by applying the Tukey's post-hoc test always at a 95% confidence level.

## 3. Results and Discussion

### 3.1 GC/MS analysis and antioxidant activity of *S. montana* EO

Twenty-six components were identified in *S. montana* EO (92.55% of the total EO identified) as shown in Table 1. The EO was characterized by an elevated percentage of oxygenated monoterpenes (61.75%), similarly to that found for *Laurus nobilis* EO [15]. The major components were thymol (28.36%), carvacrol (17.45%), and *p*-cymene (10.91%). Identical results have been reported by other authors [22,23]. However, there were differences in the percentage of most of the other constituents when compared with other findings. For example, linalool was about 2.75%, while Prieto *et al.* [24] found just some traces of this monoterpenoid in *S. montana* (Pisa, Italy) EO. Slavkovska *et al.*[22] mentioned that the chemical profile of *S. montana* EOs is quantitatively and qualitatively different depending on geographical origin and the plants' stage of development. This is consistent with the results obtained by Trifan *et al.* [25] and Nemati *et al.* [26], who mentioned that Croatian and Romanian *S. montana* EO are dominated by carvacrol with values of 84.19 and 63.40%, respectively.

Radical scavenging activity of *S. montana* EO showed an $IC_{50}$ of 40.86 ± 0.47 mg/L, while Ćavar *et al.* [27] found that *S. montana* EO from Croatia had an $IC_{50}$ of 5.49 ± 0.26 mg/mL. Antioxidant activity of EO from aromatic plants can greatly vary depending in chemical composition. Among the major components that may influence the antioxidant activity of EO, the minor constituents also can contribute acting in a synergic way. For example, the major constituents of our EO; thymol, carvacrol, and *p*-cymene are considered as active antioxidants [24,28]. Furthermore, Maestri *et al.* [29] highlighted that thymol, the major compound revealed in our *S. montana* EO, exhibited a higher antioxidant activity in soybean oil. According to several literature studies showing the antiradical activity of essential oils, the *S. montana* EO could be exploited as a source of natural antioxidants for improving the stability of lipid food systems, such as the EVOO [25,26].

### 3.2 Oxidative status of EVOO

The Chemical parameters (PV 2.50 ± 0.21; $K_{232}$ 2.23 ± 0.07; $K_{270}$ 0.14 ± 0.00) of EVOO analyzed at time zero before treatment showed that all values were lower than the limits set by the EU Regulation [30] for EVOO. The amount of pigments and TPC was also determined. The results obtained indicate a lower level of chlorophylls (2.60 ± 0.07 mg/kg) and carotenoids (1.55 ± 0.02 mg/kg) which inform about the maturity of the olives used for oil extraction. However, the amount of phenolic content was higher (1036.72 ± 0.26 mg GAE/kg) and being influenced by agronomic factors, systems extraction, and variety [31,32]. The results for PV, $K_{232}$ and $K_{270}$ of all the EVOO samples at 30, 60, and 90 days of storage under light or darkness are reported in Table 3. Data obtained in this study indicate that PV of all the EVOO samples remained always below the limits set by the EU Regulation [30] for EVOO (20 mEq $O_2$/kg), even though the PV increased during storage. This parameter, in fact, was influenced by storage time but also by storage condition ($p < 0.05$), and by the interaction between storage condition (lower under darkness), time, and EO addition ($p < 0.05$). Anterior literature data revealed a significant effect of storage condition (light, darkness) temperature, storage time, and packaging on PV value [33–36].

In comparison with the obtained results, Yildirim [37] reported that the PV of eight Turkish OO cultivar had increased and exceeded the limits after 14 months of storage in dark at room temperature

**Table 2:** Results of three-way ANOVA for the influence of *S. montana* EO bioenrichment, storage (time), fluorescent light exposition (light) and their first - and second - order interactions on the composition of Extra Virgin Olive Oil (EVOO)

| Factor | *P-value* | | | | | | |
|---|---|---|---|---|---|---|---|
| | PV | $K_{232}$ | $K_{270}$ | TPC | Chlorophyll | Carotenoids | DPPH |
| **EO** | 0.33 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |
| **Light** | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |
| **Time** | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |
| **EO × Light** | < 0.05 | 0.27 | 0.46 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |
| **EO × Time** | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 | 0.12 | < 0.05 |
| **Light × Time** | < 0.05 | 0.78 | < 0.05 | < 0.05 | < 0.05 | 0.22 | < 0.05 |
| **EO × Light × Time** | < 0.05 | 0.19 | < 0.05 | < 0.05 | < 0.05 | 0.21 | < 0.05 |

**PV**: Peroxide Value; **TPC**: Total Phenols Content

or in refrigerator. During this investigation, the PV was not affected by EO addition ($p > 0.05$). These findings agree with those obtained by Saavedra *et al.* [38], who reported that insignificant differences were detected between aromatized and non-aromatized OO with *Thymbra capitata* EO. On the other hand, Assami *et al.* [39], in their investigation, indicated an increase on PV of OO aromatized with *Carum carvi* L. EO. $K_{232}$ and $K_{270}$ can be used as indicators of the degree of OO oxidation; these coefficients are indicative of the conjugation of dienes ($K_{232}$) and the presence of the carbonyl compounds ($K_{270}$). The legislation limits for EVOO are: $K_{232}$ lower than 2.5, and $K_{270}$ lower than 0.22. Data from this investigation indicate that $K_{232}$ increased from the first month of storage with exceeding the EVOO maximum limit at 90th day, however, $K_{270}$ increased during storage but remained within the acceptable limit in all samples at the end of study. The conjugated diene systems increased and exceeded the limits at 90th day of storage in all the samples, showing a significant effect of single factors ($p < 0.05$) EO, time, and storage condition, without any effect ($p > 0.05$) due to their interactions, except for the interaction EO × time. The $K_{232}$ of EVOO stored in dark was lower than $K_{270}$ of EVOO stored under light conditions. Similar and opposite results can be found in the literature. Assami *et al.* [39] noticed a significant increase on $K_{232}$ of OO aromatized with *Carum carvi* L. EO as revealed by Asensio *et al.* [40]. These authors found that the $K_{232}$ of OO flavored with oregano spices and stored under light, increased after 126 days. Changes in $K_{232}$ values of Turkish EVOO during storage also studied by Yildirim [37]. These authors revealed that the values of $K_{232}$ increased without exceeding the maximum limit for this indice after 14 months of storage. As anticipated, the $K_{270}$ which indicates more advanced oxidation reactions remained within the acceptable limit in all samples until the end of the study. However, Caponio *et al.* [41] reported that the $K_{270}$ of OO exceeded the legal limit after two months of storage under light. $K_{270}$ increased in all OO samples showing a significant effect for all the single factors (with lower values in the presence of EO and under darkness) and their both first order and second order interactions, except for the interaction EO addition × storage conditions. Also, De la Torre-Roble *et al.* [42] found that $K_{270}$ of OO stored in dark was lower than $K_{270}$ of olive stored in light.

## 3.3 Pigments content in EVOO

The amount of chlorophylls and carotenoids decreased in all EVOO samples (Table 4), showing a significant effect ($p < 0.05$) of all the single factors (EO addition, period, and display conditions) and of their interaction on the content of chlorophylls. EVOO stored under light was found to have the lowest concentration of these pigments on the 90th day of storage, confirming the prooxidant effect of these pigments under light. Our results agree with those obtained by Gargouri *et al.* [43], who highlighted that a significant decrease in the amount chlorophylls of and carotenoids of OO stored under light has been noticed. Independently from light or darkness, addition of *S. montana* EO could slightly preserve the concentration of these pigments, in accordance with previous works [6], reporting that enrichment of OO with *Rosmarinus officinalis* and *Zataria multiflora* Boiss EOs preserve significantly the concentration of chlorophylls and carotenoids during storage.

## 3.4 Total phenols content in EVOO

The evolution of TPC of EVOO samples was analyzed during storage and the determination was repeated at 30, 60, and 90 days of storage. The results indicated that TPC underwent a remarkable decrease in all EVOO samples showing a significant influence ($p < 0.05$) of single factors (EO enrichment, time, and storage condition: dark or light), and of both their first-order and second-order interactions (Table 4). This is in agreement with the results obtained by De la Torre-Roble *et al.* [42]. The present study also indicates that EVOO enriched with *S. montana* EO clearly showed higher content in TPC at 90$^{th}$ of storage. This finding can be explained by the active antioxidant role of this EO. Rizzo *et al.*[13] reported that the amount of the TPC of OO decreased over storage under different conditions, in agreement with our findings.

Regarding the influence of light, this clearly increased the TPC degradation only in the not enriched EVOO. In the same line with the obtained data, Dabou *et al.* [44] and Gargouri *et al.* [43] studied the evolution of phenolic compounds of OO during storage under different condition and reported that the amount

**Table 3:** Evaluation of oxidative status of bioenriched EVOO (*S. montana* EO; 0.01%, v/v) during display (90 days).

| | EVOO bioenriched with *S. montana* EO | | EVOO without *S. montana* EO | |
|---|---|---|---|---|
| | Darkness | Light | Darkness | Light |
| | | Peroxide value | | |
| 30 days | 4.17 ± 0.24$^{a}$ | 4.00 ± 0.14$^{a}$ | 3.00 ± 0.12$^{a}$ | 3.00 ± 0.09$^{a}$ |
| 60 days | 10.09 ± 0.57$^{b}$ | 7.00 ± 0.11$^{c}$ | 6.44 ± 0.33$^{c}$ | 11.00 ± 0.63$^{b}$ |
| 90 days | 11.00 ± 0.11$^{b}$ | 13.00 ± 0.45$^{d}$ | 10.00 ± 0.26$^{b}$ | 15.00 ± 0.62$^{e}$ |
| | | $K_{232}$ | | |
| 30 days | 2.28 ± 0.11$^{f}$ | 2.51 ± 0.01$^{d}$ | 2.64 ± 0.06$^{acd}$ | 2.81 ± 0.03$^{abe}$ |
| 60 days | 2.58 ± 0.11$^{c}$ | 2.75 ± 0.07$^{abc}$ | 2.69 ± 0.12$^{abcd}$ | 2.91 ± 0.19$^{be}$ |
| 90 days | 2.75 ± 0.01$^{abc}$ | 2.82 ± 0.02$^{abe}$ | 2.74 ± 0.08$^{abc}$ | 2.99 ± 0.21$^{e}$ |
| | | $K_{270}$ | | |
| 30 days | 0.14 ± 0.00$^{bc}$ | 0.20 ± 0.00$^{d}$ | 0.16 ± 0.00$^{ac}$ | 0.23 ± 0.01$^{e}$ |
| 60 days | 0.14 ± 0.00$^{b}$ | 0.20 ± 0.00$^{d}$ | 0.17 ± 0.00$^{a}$ | 0.24 ± 0.00$^{ef}$ |
| 90 days | 0.17 ± 0.00$^{a}$ | 0.26 ± 0.01$^{g}$ | 0.18 ± 0.00$^{a}$ | 0.25 ± 0.01$^{fg}$ |

Values are reported as means ± S.D. of replicates (n = 3). Different letters (a-g) in a column, under the same parameter, indicates means are significantly different according to ANOVA and Tukey's post-hoc test ($p < 0.05$).

**Table 4:** Evaluation of total phenols (expressed as GAE gallic acid equivalents), total carotenoids and chlorophyll content of EVOO, with or without *S. montana* EO (0.01%, v/v) stored under darkness or fluorescent light conditions from 30 to 90 days

| | EVOO enriched with *S. montana* EO | | EVOO without *S. montana* EO | |
|---|---|---|---|---|
| | Darkness | Light | Darkness | Light |
| | | Total phenols ($mg_{GAE}$/kg) | | |
| 30 days | 992.93 ± 3.67$^{l}$ | 945.85 ± 1.75$^{k}$ | 847.45 ± 1.55$^{j}$ | 685.96 ± 2.26$^{i}$ |
| 60 days | 541.43 ± 1.96$^{h}$ | 288.13 ± 2.54$^{e}$ | 456.68 ± 2.40$^{g}$ | 329.56 ± 0.77$^{f}$ |
| 90 days | 237.28 ± 1.88$^{d}$ | 221.28 ± 2.03$^{c}$ | 216.57 ± 1.97$^{b}$ | 141.24 ± 0.56$^{a}$ |
| | | Chlorophyll (mg/kg) | | |
| 30 days | 2.51 ± 0.01$^{e}$ | 1.42 ± 0.01$^{d}$ | 1.19 ± 0.05$^{c}$ | 1.14 ± 0.07$^{c}$ |
| 60 days | 0.96 ± 0.05$^{b}$ | 0.84 ± 0.01$^{b}$ | 0.84 ± 0.07$^{b}$ | 0.79 ± 0.05$^{b}$ |
| 90 days | 0.50 ± 0.00$^{a}$ | 0.40 ± 0.01$^{a}$ | 0.58 ± 0.02$^{a}$ | 0.37 ± 0.02$^{a}$ |
| | | Carotenoids (mg/kg) | | |
| 30 days | 1.29 ± 0.05$^{c}$ | 1.245 ± 0.06$^{c}$ | 1.21 ± 0.05$^{c}$ | 1.05 ± 0.07$^{e}$ |
| 60 days | 0.85 ± 0.07$^{b}$ | 0.88 ± 0.02$^{b}$ | 0.79 ± 0.02$^{b}$ | 0.64 ± 0.00$^{a}$ |
| 90 days | 0.66 ± 0.04$^{a}$ | 0.64 ± 0.05$^{a}$ | 0.61 ± 0.04$^{ad}$ | 0.51 ± 0.01$^{d}$ |

Values are reported as means ± S.D of replicates (n = 3). Different letters (a-l) in a column, under the same parameter, indicates means are significantly different according to ANOVA and Tukey's post-hoc test ($p < 0.05$).

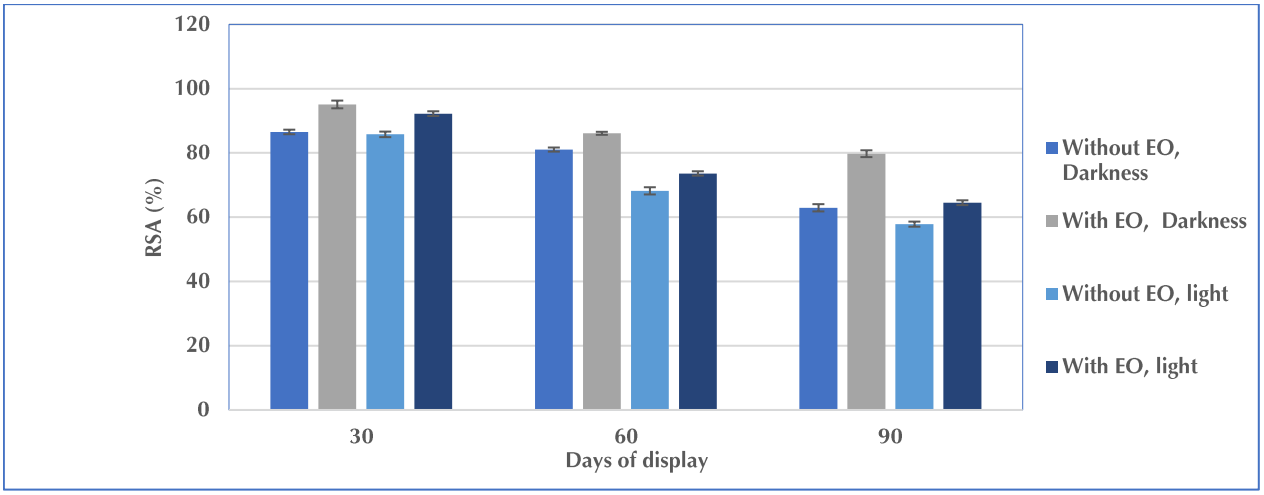


**Figure 2:** Antioxidant activity of EVOO enriched with *S. montana* EO (0.01%, v/v) during display

of the total phenol of OO stored under light decreased significantly. Since the protective effect of the addition of *S. montana* EO was observed under both storage conditions, it can be assumed that *S. montana* EO may provide some protection against both photo-oxidative and auto-oxidation degradation. According to the results obtained by Sousa *et al.* [5], flavored OO, stored at room temperature in the dark did not show any significant effect against oxidation during three months of storage. Likewise, Ayadi *et al.* [4] studied the antioxidant efficacy of some aromatic plants from Tunisia (rosemary, lavender, sage, mint, basil, lemon, and thyme) for the oxidative stability of OO stored in glass vials at 60 and 130 °C for 55 days and 6 h, respectively. These authors indicated that this bioenrichment did not exert any protective effect against thermal oxidation of TPCs. Still, Issaouia *et al.* [7] highlighted that the addition of onion in OO contributed to the increase in TPC. It must be underlined that, in these studies the added herbs were not EOs and the presence of lipophilic rather than hydrophilic antioxidant compounds can make a great difference in terms of protective effect against lipid oxidation.

### 3.5 Antioxidant capacity of EVOO

The analysis of free radical scavenging activity of EVOO throughout 90 days of storage, revealed that the antioxidant potential dropped in all the samples with a significant influence of EO oil addition, storage time, and storage condition (light or darkness) as illustrated on Figure 2. The observed decrease that is likely related to the reduction in phenolic compounds. Some authors have reported that phenolic compounds appear to have significant influence on oxidative stability [41,42]. Generally, a clear positive effect was shown by both storage under darkness and EO addition. The synergistic and antagonistic action of the major and minor compounds of *S. montana* EO are responsible for the antioxidant activity. These results support earlier work by Keramat *et al.* [6] who found that EO of *Rosmarinus officinalis* and *Zataria multiflora* Boiss exhibited an effective antioxidant potential on OO as also observed by Issaouia *et al.* [7]. However, Nour *et al.* [47] observed that the addition of tomato extract (again not an EO) to OO decreased its antioxidant potential from 18.9% to 9.4%. Compared to our findings, Baiano *et al.* [48] in their investigation revealed a decrease of antioxidant potential in OO flavored by garlic, lemon, oregano, hot pepper, and rosemary after 9 months of storage. OO, to be marketed, must meet regulatory obligations that guarantee consumers the quality of the product and contribute to its traceability to fight against fraud. The aromatization of OOs can be done using different strategies and their choice affects both the acceptability and the oxidative stability of olive oil. However, the concentration of phenolic compounds in OO may depend on the geography, olive variety, the degree of maturation, etc. Hence, an appropriate strategy to ensure an optimal intake of dietary polyphenols would be to obtain OO enriched with bioactive compounds. Some OOs are characterized by not very high phenolic contents and, therefore, low oxidation stability. In this perspective, aromatization could be an alternative to extend the shelf life of these oils, obtain oils with different organoleptic characteristics [49], in addition to diversifying the commercial offer. However, these aromatized OOs do not meet the IOC definition of olive oils, so they could not be considered in the VOO categories.

## 4. Conclusions

In the current investigation, it was pointed out that the addition of *S. montana* EO may protect EVOO against both photo-oxidation and auto-oxidation, and that exposition to fluorescent light may induce important losses in the total phenols and pigment content being, then, a valid accelerating degradation factor for accelerated shelf-life studies. Evaluation of different indices of EVOO oxidation showed a significant protective effect of both EO bioenrichment and darkness on the quality profile, but interaction of the factors was not always significant. The bioenrichment of EVOO by *S. montana* EO does not affect the properties of EVOO, on the contrary, it creates synergies and the final product is preserved even longer.

Obtaining aromatized OOs can be an opportunity to improving the nutritional quality of oils, increasing their oxidative stability, developing value-added products and diversifying the market. However, the IOC has recommended that member countries take measures to prevent these products from incorporating in their labels the terms of extra virgin olive oil, VOO and OO to avoid giving confusing information to the consumer. Consequently, it is essential that food industries adopt the measures they deem necessary for these preparations to be properly labelled, in accordance with current or future regulations.

There is one major limitation in this study that could be addressed in future research. Thus, we have to explain if the enriched oil is acceptable from the point of view of the consumer. Evaluation of sensory properties of EVOO (with or without *Satureja montana* EO) should be performed.

**Author contribution:** A.T. and D.D. contributed to the conceptualization and experimental design of the study and wrote the original manuscript draft; Z.F. conducted the data analysis; G.S. contributed to writing–review and editing. All authors have read and agreed to the published version of the manuscript.

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**Ethical approval:** The experiment does not include any animal or human testing.

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