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ORIGINAL ARTICLE

Effect of *Thymus vulgaris* L. Essential Oil on Oxidative Stability of Virgin Olive Oil and Its Synergy with Citric Acid

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ABSTRACT

Background: Since usage of synthetic antioxidants has been under question due to their toxicity and possible carcinogenicity, there is still interest in developing plant-derived natural antioxidants, especially from edible plants. This study investigated the effect of *Thymus vulgaris* L. essential oil on virgin olive oil oxidation in comparison with tocopheryl acetate and butylated hydroxytoluene (BHT). Furthermore, the synergistic activities of citric acid with *natural and synthetic antioxidants* were determined.

Methods: *T. vulgaris* essential oil was added to the virgin olive oil at a concentration of 1000 ppm. The BHT and tocopheryl acetate were added to the virgin olive oil at 100 ppm concentration. Virgin olive oil samples were stored at 60°C for 16 days. The peroxide value, *p*-anisidine value, K_{232}, and K_{268} values were measured every 4 days. Changes in chlorophyll and carotenoid contents of virgin olive oil samples were determined at the beginning and end of storage period.

Results: *T. vulgaris* essential oil significantly reduced the peroxide value, *p*-anisidine value, K_{232}, and K_{268} values of virgin olive oil. The effect of *T. vulgaris* essential oil on retarding virgin olive oil oxidation was similar to the effect of BHT. By comparison, tocopheryl acetate was less effective in reducing the oxidation of virgin olive oil. Furthermore, *T. vulgaris* essential oil could preserve chlorophyll and carotenoid of virgin olive oil during storage period.

Conclusion: *T. vulgaris* essential oil can be applied to increase the oxidative stability of virgin olive oil, and can protect the chlorophyll and carotenoid of virgin olive oil.

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Introduction

Virgin olive oil (VOO) is a vegetable oil from the fruit of the olive tree (*Olea europaea* L.) obtained only by mechanical methods that retain

its nutritional and organoleptic properties. As established in EEC Regulation 1513/2001 (2001), this oil does not require additional treatment besides mechanical washing, decantation, centrifugation or filtration (1, 2). Particular fatty acid composition and significant amounts of stability- and health-promoting components, especially tocopherols and phenolic antioxidants have made VOO a very nutritionally valuable and oxidatively stable among heating oils and fats (3, 4). However, the presence of linoleic acids and small amounts of linolenic acids makes VOO susceptible to oxidation, similar to other vegetable oils. Oxidative degradation in VOO is the most important cause of negative changes, and it happens by both enzymatic and chemical reaction pathways (5).

A consumer-friendly and safe way of improving oxidative stability of edible fats and oils is to add natural antioxidative compounds (6). Herbs and spices are one of the most important targets to search for natural antioxidants. The genus *Thymus* is one of the eight most important genera within the Labiate family. Its chemical character is represented by two main classes of secondary products, the volatile essential oil (EO) and the non-volatile polyphenols (7). Oxygenated monoterpenes thymol and carvacrol, and their corresponding monoterpene hydrocarbon precursors, i.e. *p*-cymene and γ-terpinene are the major compounds of *Thymus* EO. From the standpoint of aromatics and medicine, *Thymus vulgaris* L. (Common thyme) is indeed the most important species of the genus *Thymus* and is widely used as a flavoring agent, a culinary herb and herbal medicine (7).

Many researches have evaluated the effectiveness of different natural antioxidants on the stability of vegetable oils. For instance, a report revealed that unsaponifiable matters of bene kernel and/or hull oils were able to significantly improve the resistance of VOO to the primary and/or secondary oxidations (6). Furthermore, it is reported that the effect of Ghure marc extract on reducing the oxidation of kilka oil was similar to the effect of butylated hydroxytoluene (BHT) and better than α-tocopherol (8). On the other hand, Peñalvo *et al*. reported that natural antioxidants from oregano leaves improved the oxidative stability of VOO (9).

The aim of this study was to evaluate how the *T. vulgaris* EO can contribute to the prevention of VOO oxidation in comparison with the actions of tocopheryl acetate and BHT. Also, the synergistic effect of citric acid on natural and synthetic antioxidants was investigated.

Materials and Methods

Dried aerial parts of *T. vulgaris* were purchased from a local market in Shiraz, Iran. The genus and species of *T. vulgaris* was confirmed by experts from the herbarium of biology department at Shiraz University, Shiraz, Iran. The VOO was supplied from Etka Oil Company. All chemicals that were used in this research were of analytical grade and were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA).

For extraction of EO, 50 grams of the plant sample was mixed with 500 mL of distilled water. It was hydrodistillated for 3 h using a Clevenger-type apparatus. EO sample was dried over anhydrous sodium sulphate and were stored in sealed vials at -18 °C until further use (10). For gas chromatography (GC) analysis of EO and identification of EO constituents, qualitative analysis was done using a GC (7890A, Agilent Technologies, Santa Clara, CA) which was coupled with a mass spectrometer (5975C, Agilent Technologies, Santa Clara, CA) operating at 70 eV ionization energy, 0.5 s/scan and a mass range of 35-400 atomic mass units (amu), equipped with a HP-5MS capillary column (5% Phenyl Polysilphenylene-siloxane, 30 m length, 0.25 mm internal diameter, and 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA).

One µL of the EO sample was injected into the GC/MS in split mode (split ratio: 1/100). Helium was used as the carrier gas with a flow rate of 0.9 mL/min. The injector and detector temperatures were both set at 280°C. The oven temperature was programmed to start from 60°C and was gradually heated up to 210°C at a rate of 3°C/min. Thereafter, the rate of temperature elevation became 20°C/min until 240°C was reached, whereupon the temperature was held constant for 8.5 min. The MSD ChemStation Software (G1701EA, E.02.01.1177, Agilent Technologies, Santa Clara, CA) was employed to analyze mass spectra and chromatograms.

The compounds were identified by comparing their mass spectral fragmentation patterns with those stored in the data bank (Wiley/NBS) and with mass spectral data derived from the relevant literature (10-12). Also, quantitative analysis of EO constituents was made under the same chromatographic conditions using a GC that was coupled with a flame ionization detector (FID). The relative data for percentages were obtained from the electronic integration of the chromatogram's peak areas.

Radical scavenging activity (RSA) of the EO was measured using DPPH as described by Golmakani *et al*. The IC_{50} value was established by graph plotting, which took into account what percentage of the DPPH remains against *T. vulgaris* EO concentrations (8). The ferrous ion reducing antioxidant power (FRAP) of the EO and that of the positive control

(*L*-ascorbic acid) were determined according to the method of Ardestani and Yazdanparast (13). The reducing power was measured by reducing the Fe^{3+} to Fe^{2+}. Results were expressed as mg of ascorbic acid equivalent per gram of sample.

The cupric ion reducing antioxidant capacity (CUPRAC) of the EO and the positive control (*L*-ascorbic acid) were determined according to the method of Apak *et al.* (14). Results were expressed as mg of ascorbic acid equivalent per gram of sample. For determination of oxidative stability of VOO during accelerated storage, *T. vulgaris* EO was added to the VOO at a concentration of 1000 ppm. The BHT and tocopheryl acetate were added to the VOO at 100 ppm concentration. For the control, a sample with no added antioxidants was used. VOO samples (60 ml) were kept in closed amber bottles in an incubator at 60±1°C for 16 days.

For chemical analysis of VOO samples, the PV was determined according to the official method of American Oil Chemists' Society (AOCS) (Cd 8-53) and was expressed as meq O_2 per kg oil (15). The effectiveness of the antioxidant, also known as the stabilizing effect, is defined as the induction period extension (IPE) according to eq 1. (16).

$$\text{IPE}\ (\%) = \frac{IP_s - IP_c}{IP_c} \times 100 \qquad \text{eq 1.}$$

In the equation, IP_s is the induction period (IP) of the VOO sample with the inhibitor (BHT, tocopheryl acetate, and *T. vulgaris* EO) and IP_c is the induction period of the control. The IP is estimated as the number of days required for a sample to reach a PV of 20 meq O_2/kg (8, 17). Also, the synergistic activity was calculated according to the following eq 2.

$$\text{Synergism}\ (\%) = \left(\frac{(IP_m - IP_c) - (IP_1 - IP_c) - (IP_2 - IP_c)}{(IP_m - IP_c)}\right) \times 100 \quad \text{eq 2.}$$

In this equation, IP_m is the induction period of the sample containing the combination of the inhibitor with CA, the IP_1 is the induction period of the sample containing the inhibitor without CA, and IP_2 is the induction period of the sample containing CA (18).

The AV was determined using the official method (Cd 8-53) and was expressed as mg per kg oil. (15). The Totox value (TV) indicates total oxidation value (19). The TV value was determined according to eq 3.

$$TV=2(PV)+AV \qquad \text{eq 3.}$$

The K_{232} and K_{268} extinction coefficients were determined according to the AOCS official method (ch 5-91) by measuring the absorbance of a 1% solution in isooctane at 232 and 268 nm with 1 cm pass length (15). Chlorophyll and carotenoid contents were determined at 670 and 470 nm, respectively, according to the methods described by Minguez-Mosquera *et al.* (20). All experiments were performed in triplicates. A general linear model (GLM) procedure from SAS (Statistical Analysis Software, version 9.1; SAS Institute Inc. Cary, NC, USA) was used for the comparison of mean values. The simple regression equations of the chemical variables from the storage study of VOO were calculated using excel (Version 2010, Microsoft Office, USA).

Results

The chemical composition of *T. vulgaris* EO is presented in Table 1. *T. vulgaris* EO contained oxygenated monoterpenes (thymol (26.02%) and carvacrol (19.27%), and their corresponding monoterpene hydrocarbon precursors (*p*-cymene (27.24%) and γ-terpinene (4.38%) that are considered as their major compounds, respectively. Percentage of (E)-caryophyllene, by 2.73%, was the major sesquiterpene hydrocarbon in *T. vulgaris* EO. Of the oxygenated sesquiterpenes, caryophyllene oxide (2.15%) was predominant in *T. vulgaris* EO.

The *Z. multiflora* EO reduced the concentration of DPPH° substantially, allowing IC_{50} value to reach 0.15 mg/mL. Comparison of the DPPH° scavenging activity of the investigated EO and that expressed by BHT (IC_{50}=0.03 mg/ml) showed that the examined EO possessed slightly lower scavenging activities than BHT. FRAP and CUPRAC values of *T. vulgaris* EO were 563.84±85.63 and 690.00±10.10 mg of ascorbic acid equivalents/g sample, respectively. These results indicate that *T. vulgaris* EO had a remarkable ability to donate electrons to reactive free radicals, converting them into more stable non-reactive species and terminating the free radical chain reactions.

The antioxidants that have been used can influence the formation of hydroperoxides (Figure 1a and Figure 1d). A considerable increase in the PV of the control was observed at the early stages of the storage period, but after 12 days of storage, the PV of the control began to decrease. In fact, after 12 days of storage, the formation of hydroperoxides was slower than their decomposition into secondary oxidation products. PVs measured in samples containing *T. vulgaris* EO and BHT were lower than that of the control during the storage period.

Furthermore, *T. vulgaris* EO improved the stability of VOO to a greater extent than tocopheryl acetate. BHT, *T. vulgaris* EO, and tocopheryl acetate extended the IP of VOO by 307.94%, 245.37%, and 215.61%, respectively. The PVs of samples containing natural and synthetic antioxidants with CA were lower than those without CA. The synergistic activities of CA with BHT, *T. vulgaris* EO, and tocopheryl acetate were 2.42, 2.66, and

Table 1: Chemical composition of *Thymus vulgaris* essential oil

No.	Compound	Retention index	Relative peak area (%)
1	α-Thujene	924	0.23
2	α-Pinene	932	1.24
3	Camphene	951	1.39
4	β-Pinene	975	0.16
5	Myrcene	989	1.09
6	α-Phellandrene	1004	0.30
7	α-Terpinene	1015	1.15
8	*p*-Cymene	1025	27.24
9	Limonene	1028	0.27
10	γ-Terpinene	1058	4.38
11	cis-Sabinene hydrate	1065	0.10
12	trans-Linalool oxide	1070	0.28
13	meta-Cymenene	1087	0.52
14	Linalool	1098	2.88
15	Borneol	1164	3.81
16	Terpinene-4-ol	1175	0.92
17	α-Terpineol	1189	0.74
18	Thymol	1291	26.02
19	Carvacrol	1305	19.27
20	Piperitenone	1343	0.12
21	Thymol acetate	1354	0.51
22	Carvacrol acetate	1371	0.16
23	(*E*)-Caryophyllene	1417	2.73
24	Aromadendrene	1436	0.45
25	α-Humulene	1451	0.51
26	Spathulenol	1576	1.03
27	Caryophyllene oxide	1582	2.15
28	Caryophylla-4(14),8(15)-dien-5-b-ol	1633	0.06
29	7-epi-a-Eudesmol	1668	0.29

3.39%, respectively.

AVs of VOO samples are presented in Figure 1b and Figure 1e. The AV of the control sample increased gradually after 8 days of storage. Sharp increases in the formation of secondary oxidation products in the control sample were observed after 12 days of storage. These findings support the idea that hydroperoxides start to decompose and form secondary oxidation products such as aldehydes and ketones after 12 days of storage. All natural and synthetic antioxidants significantly retarded the formation of secondary oxidation products in comparison with the control group.

In comparison with BHT, the effects of *T. vulgaris* EO and tocopheryl acetate were slightly weaker on delaying the formation of secondary oxidation products. Combinations of natural and synthetic antioxidants with CA had a synergistic effect on preventing the formation of secondary oxidation products. TVs of VOO samples are illustrated in Figure 1c and Figure 1f. The oxidation rate of samples containing *T. vulgaris* EO was similar to that of BHT. During the formation of hydroperoxides from unsaturated fatty acids, conjugated dienes and trienes are produced due to the rearrangement of double bonds. Formation of conjugated dienes and trienes in fats and oils gives rise to an absorption peak at 232 and 268 nm, respectively.

K_{232} and K_{268} values of VOO samples during the storage period are illustrated in Figure 2. The *T. vulgaris* EO significantly reduced the levels of conjugated oxidative products in comparison with that of the control. The effect of *T. vulgaris* was similar to that of BHT on delaying the formation of conjugated dienes and trienes. The tocopheryl acetate significantly reduced the K_{232} and K_{268} of VOO in comparison with that of the control, but it was less effective than *T. vulgaris* EO and BHT. These results are in agreement with the results of PV. The CA showed a minor synergistic activity with natural and synthetic antioxidants in preventing the formation of conjugated dienes and trienes.

Durations of time that were required to reach the upper legal limits of K_{232} and K_{268} for VOO samples during the storage period are presented in Table 2. As expected, the durations of time that were

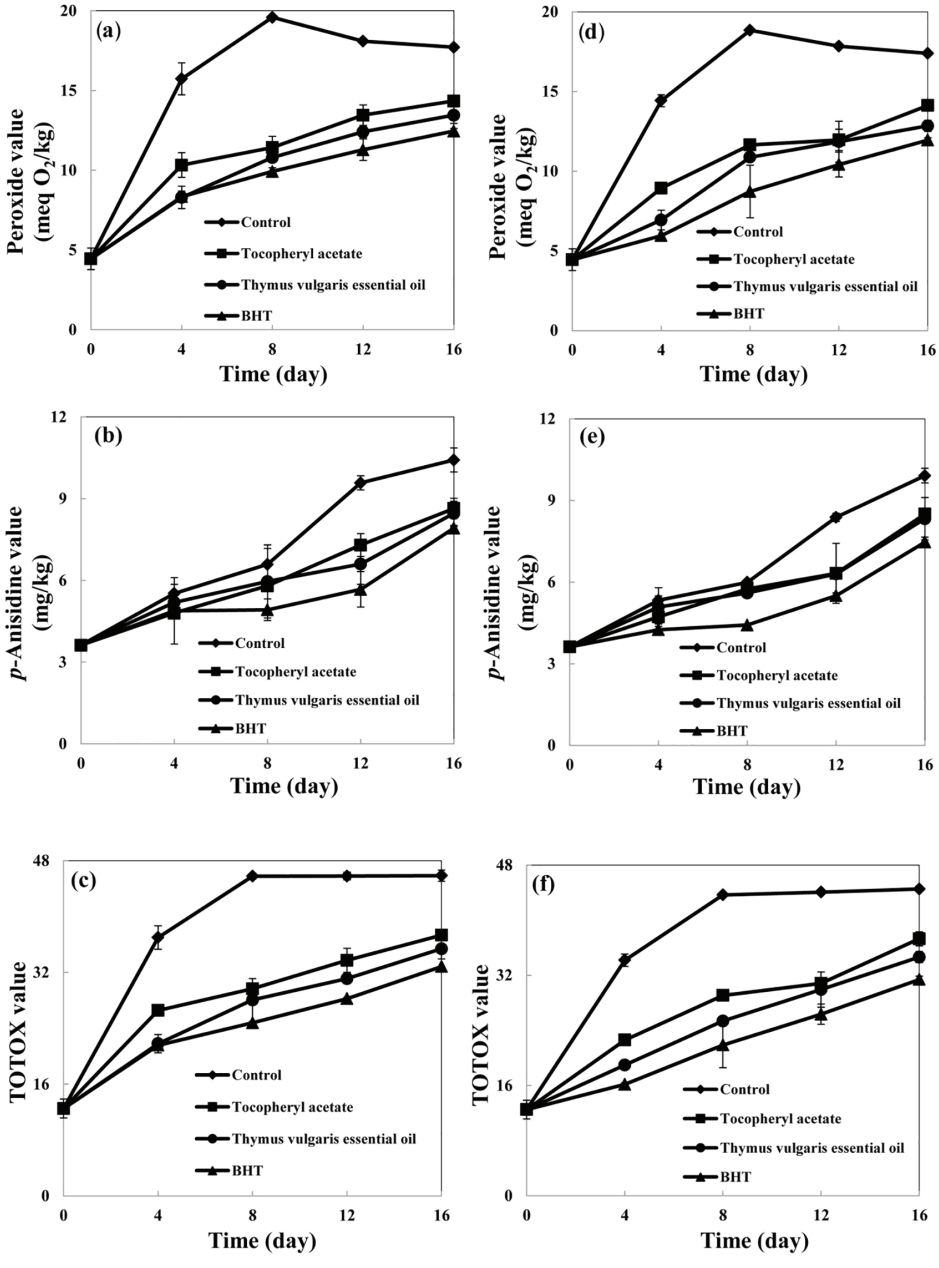

Fig. 1: Changes in peroxide values, *p*- anisidine values, and TOTOX values of virgin olive oil samples during storage at 60°C (d-f) with and (a-c) without citric acid.

required to reach the upper legal limit of K_{232} and K_{268} correlated directly with samples containing CA (R^2=0.977; y=0.326x+1.528) and also those without CA (R^2=0.941; y=0.271x+2.019). There was a direct correlation between the durations of time that were required to reach the upper legal limit of K_{232} and IP for samples with CA (R^2=0.887; y=1.086x+1.775) and those without CA (R^2=0.920; y=1.218x+0.028).

In addition, the durations of time that were required to reach the upper legal limit of K_{268} and IP correlated directly with samples containing CA (R^2=0.930; y=3.374x-3.646) and those without CA (R^2=0.941; y=4.492x-9.057). K_{268} was the first index that exceeded the upper legal limit established by the IOC for VOO (0.25). In all samples, the durations of time that were required to reach the upper legal limit of K_{232} were significantly longer than the durations required to reach the upper legal limit of K_{268}. These results indicate that both natural and synthetic antioxidants can protect conjugated dienes more than conjugated trienes.

Changes in chlorophyll and carotenoid contents of VOO samples at the beginning and end of the storage period are presented in Table 3. The chlorophyll and carotenoid contents of all VOO samples markedly decreased in response to extended storage periods, indicating that the chlorophyll and carotenoid experienced oxidative deterioration. Carotenoid decreased more than chlorophyll during the storage period. The decrease in carotenoid ranged from

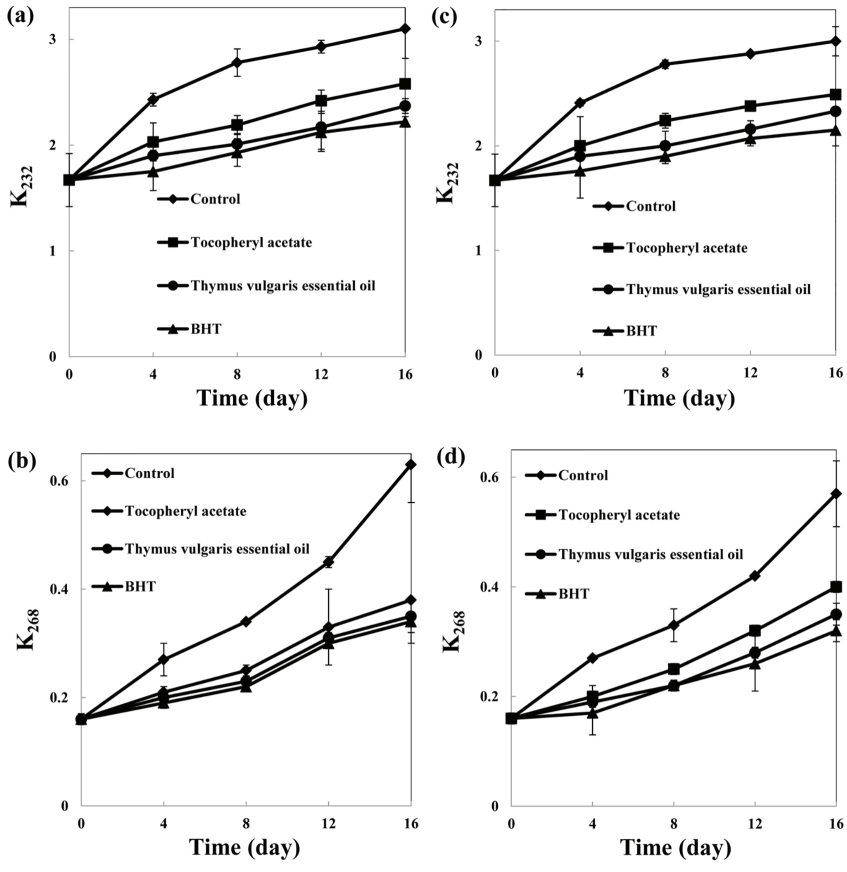

Fig. 2: Changes in K_{232} and K_{268} values of virgin olive oil samples during storage at 60°C (c, d) with and (a, b) without citric acid.

Table 2: Duration required to reach the upper legal limits of K_{232} and K_{268} for virgin olive oil samples during storage period

Treatment	K_{232} upper legal limit (day)	K_{268} upper legal limit (day)	Relative parameters		
			IP/ K_{232}	IP/ K_{268}	K_{232}/ K_{268}
Without citric acid					
Control	7.99±2.11^{e}	3.77±0.90^{b}	0.98±0.24	2.05±0.44	2.11±0.06
BHT	26.49±0.67^{ab}	8.71±0.12^{a}	1.15±0.01	3.53±0.35	3.06±0.31
Thymus vulgaris essential oil	21.85±0.56^{c}	8.25±0.59^{a}	1.19±0.08	3.17±0.17	2.66±0.21
Tocopheryl acetate	16.15±0.34^{d}	7.01±1.32b^{ab}	1.47±0.01	3.47±0.64	2.34±0.40
With citric acid					
Control	8.63±1.16^{e}	4.04±0.44^{b}	0.95±0.13	2.02±0.21	2.10±0.09
BHT	29.77±1.74^{a}	10.79±2.28^{a}	1.07±0.03	3.05±0.51	2.76±0.50
Thymus vulgaris essential oil	23.09±1.63^{bc}	9.57±1.63^{a}	1.12±0.05	2.87±0.37	2.43±0.25
Tocopheryl acetate	16.89±0.57^{d}	7.26±0.57^{ab}	1.47±0.02	3.44±0.21	2.33±0.26

*Mean±SD (n=3). In each column, means with different letters are significantly different (p<0.05). BHT: Butylated hydroxytoluene

33.40-50.18%, whereas the decrease in chlorophyll ranged from 8.13-35.58%.

Different natural and synthetic antioxidants showed various magnitudes of inhibitory effects on chlorophyll and carotenoid oxidative deterioration. BHT and *T. vulgaris* EO most significantly protected chlorophyll and carotenoid during the storage period. Therefore, *T. vulgaris* EO can be proposed as natural additives for the preservation of VOO color quality. Also, tocopheryl acetate was less effective in protecting chlorophyll and carotenoid pigments of VOO than BHT and *T. vulgaris* EO. In terms of chlorophyll and carotenoid contents, there were no significant differences between samples containing CA and those without CA at the end of the storage period.

Discussion

It was observed that *T. vulgaris* EO contained oxygenated monoterpenes and their corresponding

Table 3: Changes in chlorophyll and carotenoid contents (mg/kg) of virgin olive oil samples at the beginning and end of storage period

Treatment	Beginning of storage		End of storage		Relative reduction (%)	
	Chlorophyll	Carotenoid	Chlorophyll	Carotenoid	Chlorophyll	Carotenoid
Without citric acid						
Control	4.70±0.07^{a*}	2.05±0.06^{a}	3.02±0.00^{d}	1.02±0.11^{b}	35.58±0.90^{a}	50.18±3.62^{a}
BHT	4.70±0.07^{a}	2.05±0.06^{a}	4.27±0.07^{a}	1.33±0.01^{a}	9.06±0.13^{e}	35.01±1.67^{bc}
Thymus vulgaris essential oil	4.70±0.06^{a}	2.05±0.06^{a}	4.06±0.06^{a}	1.32±0.02^{a}	13.58±0.32^{d}	36.93±0.90^{bc}
Tocopheryl acetate	4.70±0.07^{a}	2.05±0.06^{a}	3.35±0.04^{bc}	1.25±0.04^{a}	28.68±0.16^{c}	39.24±1.08^{b}
With citric acid						
Control	4.70±0.07^{a}	2.05±0.06^{a}	3.15±0.01^{cd}	1.09±0.04^{b}	32.96±0.74^{b}	46.96±0.42^{a}
BHT	4.70±0.07^{a}	2.05±0.06^{a}	4.30±0.05^{a}	1.34±0.01^{a}	8.13±0.16^{e}	33.40±0.60^{d}
Thymus vulgaris essential oil	4.70±0.06^{a}	2.05±0.06^{a}	4.13±.02^{a}	1.33±0.02^{a}	11.99±0.95^{d}	35.04±1.01^{bc}
Tocopheryl acetate	4.70±0.07^{a}	2.05±0.06^{a}	3.48±0.18^{b}	1.26±0.01^{a}	25.92±0.65^{c}	38.81±1.20^{bc}

*Mean±SD (n=3). In each column, means with different letters are significantly different (p<0.05). BHT: Butylated hydroxytoluene

monoterpene hydrocarbon precursors, while similarly, Golmakani and Rezaei reported that thymol, carvacrol, *p*-cymene, and γ-terpinene were the major compounds of *T. vulgaris* EO (10). Our findings showed that *Z. multiflora* EO reduced the concentration of DPPHº substantially, allowing IC_{50} value to reach 0.15 mg/mL mg/mL. Also, Mishra et al. reported identically that thymol, the main component of *T. vulgaris* EO exhibited a strong free radical scavenging activity (IC_{50}=0.109 µL/mL^{-1}) (21).

We showed that the antioxidants that have been used can influence the formation of hydroperoxides. BHT, *T. vulgaris* EO, and tocopheryl acetate extended the IP of VOO by 307.94%, 245.37%, and 215.61%, respectively. The synergistic activities of CA with BHT, *T. vulgaris* EO, and tocopheryl acetate were 2.42, 2.66, and 3.39%, respectively in our study. Similarly, Hras et al. reported that the synergistic activity of rosemary extract combined with CA was 2.61% in the sunflower oil (18).

In comparison with BHT, the effects of *T. vulgaris* EO and tocopheryl acetate in the present study were slightly weaker on delaying the formation of secondary oxidation products and combinations of natural and synthetic antioxidants with CA had a synergistic effect on preventing the formation of secondary oxidation products. This anisidine value was previously shown to determine the level of aldehyde, mainly the 2-alkenals too (19).

TV indicated the primary and secondary products of the oxidation process (19). In our study, the oxidation rate of samples containing *T. vulgaris* EO was similar to that of BHT. Thymol and carvacrol were primary antioxidants, which either delayed or prevented the initiation step by reacting with a lipid-free radical or by preventing the propagation step by reacting with the peroxy or alkoxy radicals (22), thereby retarding VOO oxidation. The presence of a strongly activated methylene group in γ-terpinene can probably explain its strong behavior against lipid oxidation. The activated methylene group in γ-terpinene may compete with the activated methylene in the C-11 of linoleic acid, and thus retard linoleic acid oxidation (23).

The *T. vulgaris* EO significantly reduced the levels of conjugated oxidative products in comparison with that of the control. The effect of *T. vulgaris* was similar to that of BHT on delaying the formation of conjugated dienes and trienes. The tocopheryl acetate significantly reduced the K_{232} and K_{268} of VOO in comparison with that of the control, but it was less effective than *T. vulgaris* EO and BHT. There was a direct correlation between the durations of time that were required to reach the upper legal limit of K_{232} and IP for samples with CA and those without CA. Since linolenic acid is the most susceptible VOO fatty acid to autoxidation and its hydroperoxides undergo rapid decomposition, some of the resultant compounds have maximum absorbance at 268 nm (7). These results indicate that both natural and synthetic antioxidants can protect conjugated dienes more than conjugated trienes.

Our findings denoted that the chlorophyll and carotenoid contents of all VOO samples markedly decreased in response to extended storage periods, indicating that the chlorophyll and carotenoid experienced oxidative deterioration. BHT and *T. vulgaris* EO most significantly protected chlorophyll and carotenoid during the storage period. Therefore,

T. vulgaris EO can be proposed as natural additives for the preservation of VOO color quality. Also, tocopheryl acetate was less effective in protecting chlorophyll and carotenoid pigments of VOO than BHT and *T. vulgaris* EO. Chlorophylls can play an important role in the oxidative stability according to their antioxidant nature in the dark and their pro-oxidant activity in the light (24). Also, carotenoids can act as primary antioxidants by trapping free radicals or as secondary antioxidants by quenching singlet oxygen (25).

Conclusion

The objective of this study was to investigate the effect of *T. vulgaris* EO on the oxidative stability of VOO in comparison with BHT and tocopheryl acetate. Also, the synergistic activity of CA was investigated on natural and synthetic antioxidants. The results showed that *T. vulgaris* EO was significantly effective in retarding the oxidation of VOO during the storage period. The function of *T. vulgaris* EO in retarding VOO oxidation was as effective as BHT; however, the EO was more effective than tocopheryl acetate in the same sense. Also, CA exhibited a synergistic activity with natural and synthetic antioxidants. In conclusion, *T. vulgaris* EO could be recommended to be used as a potential source of natural antioxidants for improving the oxidative stability and to preserve the color of VOO.

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Conflict of Interest

None declared.

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