Hindawi
Journal of Food Quality
Volume 2022, Article ID 1527289, 7 pages
https://doi.org/10.1155/2022/1527289

Research Article

The Role of Thyme (*Zataria multiflora* Boiss) Essential Oil as Natural Antioxidant on the Lipid Oxidation in Mayonnaise

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Received 23 January 2022; Revised 6 March 2022; Accepted 29 March 2022; Published 13 April 2022

Academic Editor: Mohammad Jouki

Nowadays, essential oils are considered substitutes for synthetic additives in food products. Since lipid oxidation is the main chemical process affecting mayonnaise deterioration, in this research, the antioxidant activity of essential oil of thyme (*Zataria multiflora* Boiss) was determined for oxidative stability of treated mayonnaise (homogenized) during 6 months of storage. The antioxidant activities of the essential oil of thyme (0–150 μg/g) were investigated by the DPPH method. Then, the efficiency of this essential oil (144.4 μg/g) as a natural antioxidant in mayonnaise was studied by following analysis: peroxide, anisidine, Totox, and thiobarbituric acid. GC analysis of the essential oil resulted in the identification of forty compounds. The essential oil is characterized by a high number of monoterpenes such as thymol and carvacrol. Regarding antioxidation, the investigated essential oil strongly reduced the DPPH radical ($IC_{50} = 144.4\ \mu$g/ml). This study confirms that the essential oil of thyme possessed antioxidant properties *in vitro*. The results showed that the treatments containing essential oil and TBHQ significantly reduced the oxidation ($p < 0.05$), while the control sample was oxidized faster. The essential oil had a significant effect on taste, odor, and overall acceptance, but no significant difference was observed in color and texture. The results of the present experiments suggest that essential oil of thyme (*Z. multiflora*) can be used as a source of natural antioxidant for the application in food industries to prevent lipid oxidation particularly lipid-containing foods such as mayonnaise. Therefore, it can used as a natural antioxidant and flavoring compound in foods such as mayonnaise.

1. Introduction

Oxidation is the most important cause of spoilage of oily foods such as mayonnaise, causing off odor and taste [1]. The studies show that the oxidation mechanism in multiphase systems (emulsions) is much more complex than in single-phase systems. Thus, the exact mechanism of oxidation in complex food emulsions such as mayonnaise is not fully understood. Because emulsions have at least three phases, the aqueous phase, the oil phase, and the interface phase between water and oil, oxidation will occur in one of these three phases [2].

Antioxidants can be used during production to minimize oxidative damage. Synthetic antioxidants such as BHA, BHT, and TBHQ, which are widely used in food products, have adverse effects on human health [3, 4]. Therefore, strong antioxidants with less toxicity and greater effectiveness in the food industry is a serious necessity [4–6]. There is evidence for reduction of cardiovascular disease and cancer associated with natural antioxidants [6].

One of the best sources of natural antioxidants is the phenolic compounds in plant essential oils [7, 8]. There are many plants that have great biological value but have not yet been discovered in the food industry. Antioxidant effects of natural substances such as essential oils and extracts of berries [9], green tea [10], raisins [11], olives [12], and grape seeds [13] in oil-in-water emulsions have been previously studied.

The antioxidant effects of extracts and essential oils such as *Z. multiflora* and *Satureja hortensis* [14], aloe vera extract [15], black rice extract [16], black pepper and basil extract [17], ginger powder [18], corn extract [4], yarrow essential oil [2], and tangerine (*C. reticulata* var. Arrayana) peels [19] in mayonnaise have been previously investigated and reported that extract and essential oils can be used in mayonnaise as a natural antioxidant and can replace chemical antioxidants.

One of the other aromatic plants is *Z. multifora* Boiss, which has many medicinal and therapeutic properties and can be used as an antimicrobial and antioxidant agent in food products [14].

Due to the high consumption of mayonnaise and the presence of synthetic additives in this product and the adverse effects of these compounds on the health of consumers, the need to use compounds with less harm is a serious necessity. The aim of this study is the investigation of the antioxidant effect of *Z. multifora* Boiss essential oil and delay the oxidation process in mayonnaise.

2. Materials and Methods

2.1. Plant Collection. The flowering of *Z. multiflora* plant was prepared in the summer 2019 and was identified by the botanical herbarium (no. 23459). It was then dried in the shade and powdered in a mill (Mulinex, Spain) and stored in the freezer.

2.2. Extraction of Essential Oil. The essential oil was extracted by using Clevenger (Ashk shishe, Iran) and was dehydrated by sodium sulfate and stored in dark, closed jars at 4°C [20].

2.3. GC-MS Analysis. The essential oil was analyzed using GC-MS (Model YL6100, Young lin company) equipped with a flame ionization detector (FID). It was fitted with a BPX 70 capillary column (30 m × 0.25 mm i.d., 0.25 μm). The initial oven temperature was 60°C, then gradually increased to 280–300°C at a 4°C/min rate. The carrier gas flow rate (Helium) was 1 ml/min. Injector and MS transfer line temperatures were set at 220 and 290°C, respectively. Column temperature was initially at 60°C, then gradually increased to 280–300°C at a 4°C/min rate. Essential oil was injected manually. The compounds were detected based on the comparison of their relative retention time (RT), retention indices (RI), and mass spectra data with those of library data of the GC-MS system, literature data (Adams, 2007), and standards of the main components and literature and National Institute of Standards and Technology (NIST, Wiley). RI was calculated manually by interpolation, using retention times of the reference *n*-alkanes (run under identical conditions) eluting immediately before and after the component in question [20].

2.4. Antioxidant Properties. The antioxidant activity of *Z. multiflora* was analyzed by the DPPH method and compared with TBHQ. First, 2 mL of DPPH ethanol solution (freshly prepared at a concentration of 0.1 mM) were added to 2 ml of essential oil at concentrations of 0, 10, 50, 100, and 150 μg/g and was stored in the dark place for 30 min at 20°C, and the absorbance of the samples was measured using a UV/VIS spectrophotometer (model 7220G-9200, Beijing Beifen-Ruili, China) at a wavelength of 517 nm. TBHQ was used as the reference. Using following formula, the radical scavenging capacity (RSC) was calculated [20, 21].

$$RSC\,(\%) = 100 \times \left(A_{\text{blank}} - \frac{A_{\text{sample}}}{A_{\text{blank}}} \right). \quad (1)$$

The IC_{50} value, which represented the concentrations of the essential oil that caused 50% inhibition, was determined by linear regression analysis from the obtained RSC values.

2.5. Mayonnaise Preparation. Based on the antioxidant activity of the essential oil in the DPPH test, the essential oil at IC_{50} (144.4 μg/g) was added to the oil phase of mayonnaise. The control sample was containing TBHQ at 0.12 mg/ml concentration [22].

Mayonnaise was prepared by mixing the oil phase including antioxidant-free soybean oil (65%), water, vinegar, and eggs and powdered ingredients including salt, sugar (crystal), citric acid, mustard powder, xanthan, carboxymethylcellulose, benzoate sodium, and potassium sorbate. The mayonnaise samples were divided into three groups and packed in glass containers and covered with aluminum foil to prevent light from entering and kept in the refrigerator (4°C) until the experiments.

2.6. Mayonnaise Oxidation. The oxidation of mayonnaise was determined in 6 months. The oil was extracted according to the cold extraction method [23]. The peroxide value, thiobarbituric acid, and anisidine were determined according to AOCS method nos. Cd 8-53, Cd 19-90, and Cd 18-90, respectively [24–26]. To calculate the peroxide value, 5 g of oil extracted in 25 ml of the chloroform acetic acid mixture was dissolved in a ratio of 2 : 3. Then, 1 ml of potassium iodide saturated solution was mixed into the mixture and placed in the dark for 10 min. In the next step, 30 ml of distilled water was added, and the mixture was titrated with 0.01 M in the presence of starch solution with sodium thiosulfate. To calculate TBA, 5 g of oil extracted was mixed with 15 ml of 20% TCA solution and homogenized for 1 min and then filtered. 2 ml of the mixture was transferred to a test tube and mixed with 2 ml of 0.02 M aqueous 2-thiobarbituric acid. Then, the mixture was placed at 90°C for 30 min to react. After cooling, the absorbance of the samples was determined at 532 nm. The TBA value was measured as mg malondialdehyde (MDA)/kg sample. To calculate the

Table 1: Chemical compounds (%) of essential oil of *Z. multiflora*.

No	Compound	RI	Rt (min)	Relative peak area (%)
1	α-Thujene	924	1.533	0.1532
2	α-Pinene	932	4.232	3.933
3	3-Octanone	984	5.620	3.203
4	Myrcene	988	6.527	1.202
5	α-Terpinene	1014	6.849	10.87
6	p-Cymene	1020	7.931	3.239
7	γ-Terpinene	1054	13.060	0.4015
8	Linalool	1095	15.422	0.5058
9	Carvacrol methyl ether	1241	17.863	0.9719
10	Thymol	1289	18.363	26.93
11	Carvacrol	1298	18.776	42.22
12	Eugenol	1361	23.088	1.268
13	Carvacrol acetate	1370	29.882	2.846
14	β-Caryophyllene	1417	40.757	2.253

Table 2: The antioxidant activity of *Z. multiflora* essential oil and TBHQ.

Concentration (μg/ml)	EO	TBHQ
0	1.04 ± 0.60^{E}	1.84 ± 0.73^{E}
10	4.25 ± 1.0^{D}	49.84 ± 0.97^{D}
50	10.58 ± 1.34^{C}	76.36 ± 1.08^{C}
100	25.64 ± 2.79^{B}	89.98 ± 0.56^{B}
150	57.13 ± 0.7^{A}	97.36 ± 0.48^{A}
IC_{50}	144.4	37.2

Mean ± SD (standard deviation) with different letters differ significantly ($p < 0.05$).

anisidine value, 1 g of oil extracted was dissolved in isooctane and its absorbance was determined at 350 nm. After that, 1 ml para-anisidine was added to the mixture and its absorbance was determined [27]. The Totox index was calculated using the mathematical formula (2% peroxide) + anisidine number [28].

2.7. Statistical Analysis. All experiments were performed in a completely randomized design with three replications, and the mean data were compared with the Duncan test at the level ($p < 0.05$) with the SAS V 9.1 software.

3. Results and Discussion

3.1. Chemical Composition of Essential Oil. The yield of *Z. multiflora* Boiss essential oil was 1.64% (w/w) based on the dry weight. A total of 14 components were identified by the GC method (Table 1). Based on the results, carvacrol, thymol, α-terpinene, α-pinene, 3-octanone, and carvacrol acetate formed the major constituents of the essential oil.

3.2. Antioxidant Activity. Table 2 shows the antioxidant activity of *Z. multiflora* Boiss essential oil at different concentrations (0–150 μg/g) compared to synthetic antioxidants TBHQ.

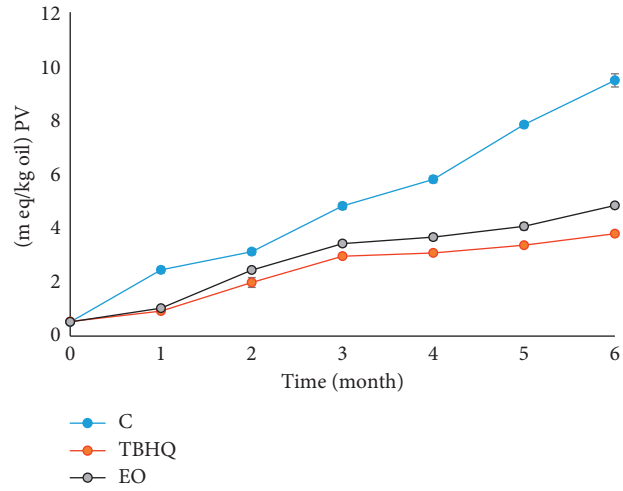

Figure 1: The peroxide value (meq/kg) in different treatments of mayonnaise during 6 months. EO: sample containing *Z. multiflora* essential oil; TBHQ: sample containing synthetic antioxidant TBHQ; C: control sample (control).

IC_{50} is inversely related to the antioxidant activity of the essential oil. As it is known, there is a direct relationship between the concentration of essential oil and its radical inhibitory effect, and with increasing the concentration of essential oil, its antioxidant effect increases. In the case of TBHQ, an increase in inhibitory potency was observed at high concentrations. TBHQ also showed more antioxidant activity compared to an essential oil ($p < 0.05$). High levels of thymol (26.93%) and carvacrol (44.22%) in essential oil are probably responsible for its strong antioxidant effects (Table 1). Also, phenolic compounds such as carvacrol, thymol, eugenol, and terpinene probably had the antioxidant activity in this essential oil. The antioxidant activity of compounds such as pinene, terpinene, and thymol has been previously reported [29]. The results of the previous research also confirm the strong antioxidant effects of *Z. multiflora* essential oil [12, 30–32].

3.3. Peroxide Value. As shown in Figure 1, *Z. multiflora* essential oil significantly delayed the formation of hydroperoxides ($p < 0.05$). Although at the production time, the peroxide value of the treatments did not differ significantly (0.52 meq/kg), the trend of changes over time showed a different pattern ($p < 0.05$). In all samples, the peroxide value increased gradually with time to the end of the test period. For example, in the sixth month, the peroxide value of the control sample was eight times the production time (9.47 meq/kg). The control sample had the highest oxidation rate and the sample containing TBHQ had the lowest oxidation rate ($p < 0.05$).

Hydroperoxides are the primary products of lipid oxidation and their amount increases and then decreases during oxidation [33]. The increase in the peroxide value over time is due to the increase of oxidation with storage time. The peroxide value of the samples containing essential oil and TBHQ was lower than the control sample ($p < 0.05$). In the

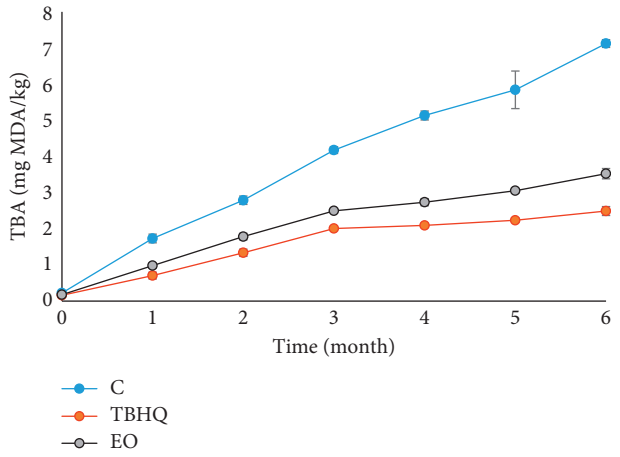

Figure 2: The thiobarbituric acid index (mg MDA/kg) in different treatments of mayonnaise during 6 months. EO: sample containing *Z. multiflora* essential oil; TBHQ: sample containing synthetic antioxidant TBHQ; C: control sample (control).

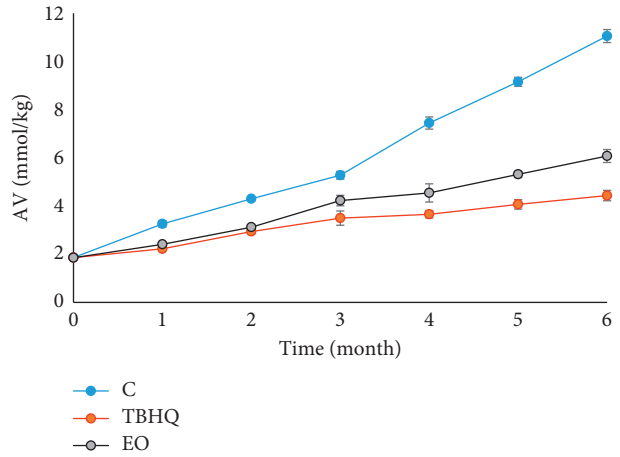

Figure 3: The anisidine index (mmol/kg) in different treatments of mayonnaise during 6 months. EO: sample containing *Z. multiflora* essential oil; TBHQ: sample containing synthetic antioxidant TBHQ; C: control sample (control).

early days, all treatments containing natural and synthetic antioxidants were able to maintain their antioxidant power and had a better antioxidant effect than the control sample. Therefore, the difference between the treatments and the control sample was obvious in the early days. With increasing the storage time, the difference between the treatments became more pronounced. As shown in Figure 1, the control sample showed the highest peroxide value during the storage period (9.47 meq/kg). This is because soybean oil in mayonnaise contains high levels of polyunsaturated fatty acids such as linoleic acid (59.59%) and linolenic acid (7%). In general, the rate of oxidation increases with an increasing degree of unsaturation of oils. Because mayonnaise is an oil-in-water emulsion and its oily phase is in contact with a large area of water, it is very prone to oxidative damage. On the other hand, the aqueous phase in mayonnaise emulsion carries high amounts of oxygen, which increases oxidation [34]. As mentioned earlier, some essential oil compounds, including thymol and carvacrol, have strong antioxidant effects. Polyphenols can trap free radicals, thus ending the cycle of oxidation reactions [4, 16]. Similar results in the use of other natural antioxidants such as extracts and essential oils have been reported in mayonnaise including corn extract [4] and brown rice glutenin extract [16].

3.4. Thiobarbituric Acid. The peroxide value is an indicator of the primary oxidation products and does not specify the secondary production of oxidation. Therefore, determining the TBA index is necessary [35]. As it is known from Figure 2, there was no significant difference between the thiobarbituric acid of essential oil, TBHQ, and the control sample immediately after production, but a significant difference was observed at the level of 0.05. The TBA index during storage in all samples containing antioxidants was significantly lower than the control sample ($p < 0.05$), which indicates the protection of mayonnaise from oxidation by antioxidants. The thiobarbituric acid index of the samples increased significantly over time ($p < 0.05$). The thiobarbituric acid index of the control sample was increased from 0.22 to 7.12, the sample containing TBHQ from 0.16 to 2.49, and sample containing thyme essential oil from 0.18 to 3.53 mg MDA/kg in the sixth month.

The results show that *Z. multiflora* essential oil in mayonnaise had an antioxidant effect and acted well in preventing the formation of malondialdehyde, which is attributed to the accumulation of phenolic compounds (Table 1). The trend of TBA index changes was similar to the peroxide value (Figures 1 and 2). As shown in Figure 2, a rapid increase in the TBA index was observed in the control sample. The maximum TBA was observed in the sixth month (7.12 mg/kg). By comparing the TBA index between the samples, it can be concluded that the samples containing antioxidants were less affected by oxidation than the control sample. In addition, with increasing storage period, a continuous increase in the TBA index was observed for all samples. Therefore, time has an important effect on the oxidation of mayonnaise so that the level of TBA in all samples reached its highest level in the sixth month.

It can be said that *Z. multiflora* essential oil was able to compete with synthetic antioxidants. Probably, the reason for the protection of mayonnaise containing essential oil from oxidation is the presence of terpenes and phenolic compounds such as thymol, carvacrol, pinene, and terpinene. Similar results have been obtained in previous studies. Tananuwong and Tewaruth [16] showed that the addition of brown rice glutenin extract to mayonnaise, due to its phenolic compounds, delayed the increase of the TBA index. Ahmadi-Dastgerdi et al. [2] achieved similar results and stated that yarrow essential oil in mayonnaise can delay the formation of primary and secondary oxidation products.

3.5. Anisidine Index. The results of the anisidine index were similar to the results of the peroxide index and TBA (Figure 3). The anisidine index of the samples was not

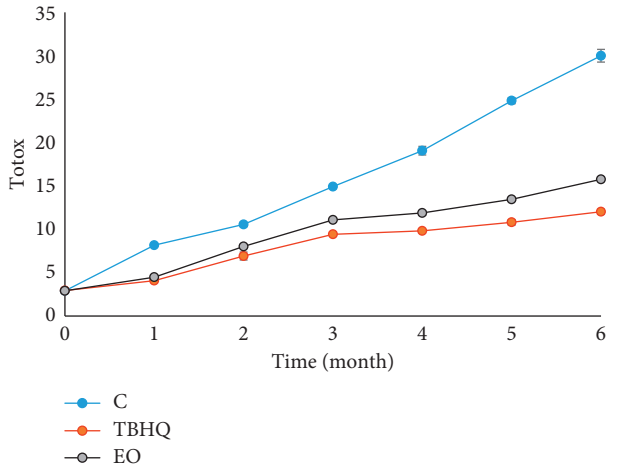

Figure 4: Totox index in different treatments of mayonnaise during 6 months. EO: sample containing *Z. multiflora* essential oil; TBHQ: sample containing synthetic antioxidant TBHQ; C: control sample (control).

significantly different after production ($p < 0.05$) but over time the results increased significantly. The anisidine index in mayonnaise containing *Z. multiflora* essential oil was significantly lower than the control sample during the six-month ($p < 0.05$). The anisidine index of the control sample reached 11.05 mmol/kg in the sixth month. The anisidine index of the sample containing TBHQ increased from 1.85 to 4.43 mmol/kg, and the sample containing essential oil increased from 1.85 to 6.8 mmol/kg in the sixth month.

All treatments showed the lowest and highest values of the anisidine index at time zero and the end of the sixth month, respectively. That is, the oxidation changes of the oil continued with increasing storage time, so the amount of the anisidine index in the samples also increased and reached a maximum at the end of the storage period. In comparison between *Z. multiflora* essential oil and TBHQ, the highest antioxidant effect was observed by TBHQ ($p < 0.05$). The highest level of anisidine index was also observed in the control sample. The increase in the anisidine index in the control sample is probably related to the effects of oxidation. Li et al. [4] also measured the primary and secondary oxidation products and showed that corn extract could protect mayonnaise from oxidation.

3.6. Totox Index. The Totox index of the samples is shown in Figure 4. Among the treatments, the control sample had the highest oxidation rate (29.99) and the sample containing TBHQ (12.02) and essential oil (15.76) had the lowest oxidation rate.

Mayonnaise containing *Z. multiflora* essential oil prevented the formation of primary and secondary oxidation products. It can be concluded that *Z. multiflora* essential oil delays the oxidation process in mayonnaise. The ability of essential oils to delay oxidation is related to the composition of essential oils. High levels of thymol (26.93%) and carvacrol (42.22%) in essential oil are probably the reason for its strong antioxidant effects (Table 1). Also, the combination of monoterpene and sesquiterpene hydrocarbons (including pinene, terpinene, and monoterpene alcohols) are likely to be active receptors in the essential oil. These compounds act synergistically with each other and with other compounds. The evaluation of natural antioxidants in oil-in-water emulsions is complex due to the interface between oil and water. Frankel et al. [36] showed that the effect of lipophilic and hydrophilic antioxidants depends on the type of substrate, physical state (oil and emulsion), antioxidant concentration, time, temperature, and method used to evaluate the antioxidant activity. For example, hydrophilic antioxidants dissolve in the aqueous phase and cannot protect the oil in emulsions in the oil-water interface. This is probably due to the comfortable position in the oil-water interface [5], so the antioxidant position are effective in preventing the oxidation of oil emulsions in water.

4. Conclusion

In this study, *Z. multiflora* Boiss essential oil had antioxidant activity *in vitro* and mayonnaise. It significantly retarded the formation of primary (peroxide value) and secondary (TBA and anisidine value) products of oxidation in mayonnaise, so it can be a promising source of natural antioxidant resources. Therefore, it is possible to reduce the consumption of chemical additives by using *Z. multiflora* Boiss essential oil to play a role in providing more health in society.

Data Availability

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

Conflicts of Interest

The authors have no conflicts of interest to report.

Acknowledgments

The authors are grateful to the respected authorities of the research lab at Science and Research Branch, Islamic Azad University, Isfahan, Iran.

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