Application of *Satureja hortensis* L. and *Zataria multiflora* Boiss. Essential Oils as Two Natural Antioxidants in Soybean Oil During Microwave Heating

Fathi A (M.Sc.)[1], Sahari MA (Ph.D.)[1]*, Zangiabadi M (M.Sc.)[1], Barzegar M (Ph.D.)[1]

1- Department of Food Technology, College of Agriculture, Tarbiat Modares University, Tehran, Iran
* Corresponding author: Food Technology Department, College of Agriculture, Tarbiat Modares University, Tel: +98 – 21 – 48292328, Fax: +98- 21- 48292200
E-mail: sahari@modares.ac.ir

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Abstract

Background: Autoxidation is considered to be the main route of edible oil deterioration, which produces undesirable odors and flavors during storage and heating. The unpleasant flavor of oxidized oil has been attributed to primary and secondary products of unsaturated fatty acids. The microwave heating is a rapid method when compared with other methods of heating.

Objective: In this research, the effect of microwave heating on the oxidative stability of soybean oil amended with either of *Satureja hortensis* L. (SHEO) and *Zataria multiflora* Boiss (ZMEO) essential oils was evaluated and compared with synthetic antioxidant (butylated hydroxyl toluene= BHT; 200 ppm).

Methods: Soybean oil containing with SHEO (200 and 1000 ppm), ZMEO (200 and 1000 ppm) and BHT (200 ppm) were heated using microwave oven (600 W) for 25 min and spectrophotometer was used to determine UV absorption. Peroxide value (PV), conjugated dienes (CD) and conjugated trienes (CT) indices were measured.

Results: Statistical results showed that PV after 25 min were increased in all treatments and the stability of soybean oil treated with synthetic antioxidant (BHT, 200 ppm) was higher than oil treated with the studied essential oils (SHEO and ZMEO, 200 and 1000 ppm). Diene and triene indices increased during the heating. There was a positive correlation between PV, diene and triene indices during the process of microwave heating.

Conclusion: The two essential oils (SHEO and ZMEO, in concentrations of 200 and 1000 ppm) showed fairly good antioxidant activities as compared with BHT (200 ppm) in soybean oil control sample (refined- bleached- deodorized soybean oil without any additives).

Keywords: Natural antioxidant, Microwave, Essential oil, *Satureja hortensis*, *Zataria multiflora*, Soybean oil

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Introduction

Autoxidation is the main reaction in degradation of food lipids, and heating has an important role in both industrial and home processing, also it has economic, nutritional, taste and flavor, safety and preservation aspects for foodstuff [1]. Microwave heating produces heat based on interaction of electromagnetic field and chemicals in foods through friction and molecular stimulation; so, microwave heating is a faster method relative to other methods [2]. Few studies have been conducted on chemical and physical changes of heated oils by microwave, and most of them have considered the effect of microwave on nutritional characteristics of foods. Changes in long chain fatty acids due to thermal reactions requires a long time in ambient conditions while such changes occur rapidly in microwave. On the other hand, studies have shown that microwave heating for 8 to 10 min reduces levels of tocopherols in linseed, olive and palm oils, and increasing the heating time also increases peroxide values [3].

Food lipids with a high ratio of unsaturated fatty acids (UFA), especially polyunsaturated fatty acids (PUFA), have high oxidation sensitivity [1]. Oxidation of oils not only causes off odor and off flavor and color changes but also reduces nutritional quality and safety of products resulting in harmful effects on human health [4].

Off odor and off flavor of oxidized oils are results of formation of primary and secondary oxidation products of unsaturated fatty acids. As a result of oxidation reactions of unsaturated fatty acids by displacement of diene and polyene bonds due to isomerization and conjugation, absorption will increase in ultra violet (UV), and thereby the conjugated dienes and trienes formed show high absorption in 232 and 270 nm, respectively [2].

Synthetic antioxidants such as butylated hydroxyl anisole (BHA), BHT and tert butyl hydro quinine (TBHQ) are widely added to foodstuff to avoid or reduce oxidative effects. Recent studies have indicated that such components can cause hazards such as carcinogenesis and toxicosis. Therefore, there has recently been a high interest to use natural antioxidants present in spices and plant materials as promising substitutes [4].

Essential oils contain complex odorous volatile components, which are soluble in oil [5]. Essential oils are used in food and pharmaceutical industries because of their antifungal, antibacterial, antioxidant and other effects [6, 7].

Satureja hortensis L. belongs to *Lamiaceae* family (Labiatae**;** minths) and is well known in Iranian traditional medicine [8]. *S. hortensis* is used in treating diarrhea, cramps, nausea and as muscle pain reliever [9]. The volatile oil, oleoresin and extracts of *S. hortensisl* L. are used as seasoning in the food industry [10]. In addition, most essential oils have antimicrobial, activities due to their high phenolic contents [11, 12].

Zataria multiflora Boiss (*Labiatae*) is an herbal plant with several thin, hard and highly ramified stems from family. The essential oil of *Zataria multiflora* Boiss is extracted from the flowered browses of the plant, having the compounds with important pharmaceutical, antimicrobial, and antioxidant effects [13]. Thereupon, these two essential oils were selected for their good antioxidant effect in this research.

The objective of this study was to evaluate the antioxidative effects of the essential oils of two medicinal plants (*Satureja hortensis* L. and *Zataria multiflora* Boiss essential oils: SHEO and ZMEO) on soybean oil stability under microwave treatment and its comparison with a synthetic antioxidant (BHT).

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Materials and Methods

Materials

Refined- bleached- deodorized (RBD) soybean oil without any additives was purchased from Margarine Factory (Tehran, Iran). SHEO and ZMEO were obtained from the Institute of Medicinal Plants and Natural Products Research, Karaj, Iran. Chloroform, acetic acid, potassium iodide, potassium iodate, starch, sodium thiosulfate, butylated hydroxyl toluene (BHT) and cyclohexane were purchased from Merck (Darmstadt, Germany). All chemicals were analytical grade with the highest purity available and were used without further purification. Oils were extracted by hydrodistillation of the aerial parts using Clevenger-type apparatus for 3 h [15].

GC/MS analysis of essential oil

Quantitative data were obtained from the electronic integration of the FID peak areas. GC analysis was carried out on Agilent Technologies 6890 gas chromatograph equipped with flame ionization detector (FID) and a HP-5 capillary column (30 m 0.25 mm; 0.25 um film thickness). The oven temperature was held at 50 ºC for 5 min, and then programmed at 3°C/min to 240 ºC and after that programmed at 15 ºC/ min to 300 ºC (held for 3 min) and finally reached 340 ºC (at 3 ºC min). Other operating conditions were: carrier gas, He with and a flow rate of 0.8 ml/min: injector and detector temperatures were 290 ºC and 209 ºC, respectively; split ratio, 1:10. GC/MS analysis was performed on a GC mentioned above coupled with an Agilent Technologies 5973 Mass system. The other operating conditions were the same conditions as described above, mass spectra were taken at 70 eV. Mass range was from m/z 35 - 375 amu. Quantitative data were obtained from the electronic integration of FID peak areas. The components of the essential oils were identified by comparison of their mass spectra and retention indices with those published in the literature and presented in the MS computer library [15].

Preparation of oil sample

SHEO and ZMEO were added to RBD soybean oil at two levels 200 and 1000 ppm (for investigation of eventual pro oxidation effect of these two essential oils). Synthetic antioxidant (BHT) was employed at its legal limit of 200 ppm to compare the efficacy of essential oils [15]. Oil samples (25 ml) were placed in dark brown airtight glass bottles with narrow neck and heated in a microwave oven (CE300S-TDH, Butan, Tehran, Iran) at a frequency of 2450 MHz (medium power setting, capable of generating 600 W). All oil samples were prepared in triplicate. Soybean oil sample, without antioxidant, was used as control. Oil samples were taken after every 5 min interval up to 25 min. The oxidative deterioration level was assessed by the measurement of peroxide value (PV), conjugate dienes (CD) and conjugate trienes (CT) values.

Analytical procedures

Peroxide value (PV)

Peroxide value (PV) of all treated oil samples were measured according the AOCS method Cd 8-53 (AOCS, 1997) [16].

Conjugated dienes (CD and trienes (CT)

Specific absorbance at 232 and 270 nm (foe determining. conjugated dienes and trienes, respectively) were determined spectrophotometerically at 232 and 270 nm (spectrophotometer, Scinco, Korea). Oil samples were diluted with cyclohexane to bring the absorbance within allowed range following the IUPAC method II. D. 23

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(IUPAC, 1979) [17].

Statistical analysis of experimental data

Analysis of variance was performed by ANOVA using SPSS (version 16.0, SPSS Inc., Chicago, IL, USA). Significant differences ($p < 0.05$) of means were calculated using Duncan's multiple range tests. Data are presented as mean ± standard deviation of the three replicates.

Results

Tables 1 and 2 show chemical compositions of SHEO and ZMEO, respectively, as determined by GC/MS.

Peroxide values of soybean oil control samples after heat treatment (by microwave) are given in Table 3.

Tables 4 and 5 show the amount of diene and triene formed during microwave heating.

Table 6 compares measurement of peroxide, diene and triene indices in the present study with other studies conducted in various oils together with different antioxidants in microwave conditions.

Discussion

GC/MS analysis of essential oils

As shown in Table 1 *Satureja hortensis* contains carvacrol (24.50%), thymol (23.12%), γ-terpinene (20.72%) and ρ-cymene (6.52%). Novak et al (2006) have reported the same major components for SHEO [18].

According to Table 2, the most abundant chemical components in ZMEO are carvacrol (26.08%), ρ-cymene (20.34%) and thymol (17.23%). Thymol and carvacrol, the main components of the plant essential oils, have good antimicrobial and antioxidant effects [19].

Table 1- Main composition of *Satureja hortensis* L. (%) determined by GC/MS

Components	Kovat's retention index	Retention time (min)	Composition (%)
α-Thujene	931	10.61	1.24
α-Pinene	944	10.99	2.91
Camphene	953	11.66	0.15
β-Pinene	980	12.96	0.92
Myrcene	991	13.16	1.98
α-Phellandrene	1.005	14.18	0.74
α-Terpinene	1.018	14.79	2.93
ρ-Cymene	1.026	15.36	6.52
Limonene	1.031	15.94	2.55
γ-Terpinene	1.062	16.97	20.72
Cyclopentene	1.165	22.20	0.21
Terpinene-4-ol	1.177	22.80	0.17
L-carvone	1.242	27.31	0.54
Thymol	1.290	28.87	23.12
Carvacrol	1.298	29.18	24.5
Carvacrolacete	1.371	31.88	0.75
Aromanderene	1.439	34.86	0.34
Ledol	1.500	37.12	0.19
β-Bisabolene	1.509	37.47	2.20

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Continue Table 1- Main composition of *Satureja hortensis* L. (%) determined by GC/MS

Components	Kovat's retention index	Retention time (min)	Composition (%)
α- Bisabolene	1.515	38.70	0.19
Spathullenol	1.576	40.62	0.23
Caryophyllenxie	1.581	40.85	1.70
Benzenemethanol	1.838	52.69	0.19
Total			94.99

The retention index has been calculated by injecting of normal hydrocarbon mixture (C_9-C_{26}) to DB-1 column

Table 2- Main composition of *Zataria multiflora* Boiss. (%) determined by GC/MS

Components	Kovat's index	Retention time (min)	Composition (%)
α – Thujene	934	16.82	0.18
α – Pinene	942	17.29	3.80
Camphene	959	18.11	0.20
β – Pinene	982	19.68	0.48
3-octanone	988	20.06	0.71
β -myrecene	992	20.33	1.08
3-octanol	995	20.55	0.19
α -terpinene	1020	21.85	0.44
P- Cymene	1028	22.48	20.34
Limonene	1032	22.61	0.99
1,8-cineole	1035	22.75	1.00
γ- Terpinene	1064	24.11	0.26
Trans-sabinenehydrate	1096	25.74	0.21
Linalool	1100	26.39	10.09
Borneol	1167	29.88	0.21
4-trpineol	1186	30.42	0.68
α –terpineol	1192	31.24	0.65
Thymol methyl ether	1237	33.04	1.41
Carvacrol methyl ether	1248	33.55	3.84
Thymol	1293	35.98	17.23
Carvacrol	1301	36.62	26.08
β –caryophyllene	1423	42.01	4.27
Aromadendrene	1443	42.30	1.36
Alpha-humulene	1458	43.40	0.23
Allo aromadendrene	1464	43.72	0.13
Ledene	1493	45.09	0.58
Spatulenol	1578	48.53	0.64
Caryophyllene oxide	1584	48.82	1.64
Total			98.92

The retention index has been calculated by injecting of normal hydrocarbon mixture (C_9-C_{26}) to DB-1 column

Antioxidant effect of studied essential oils on RBD soybean oil

Peroxide value is a chemical indicator that shows progress in the early stages of oil oxidation [20]. It is, however not an appropriate index to follow the effect of microwave heating on oil oxidation, because hydroperoxides are unstable after heating at

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high temperature. However, deteriorative progress is related to hydroperoxide formation, and also the hydroperoxides are not directly responsible for off flavor due to oxidative deterioration. Conjugate diene and triene measurements are an appropriate way to measure the oxidation progress in oils and are therefore considered as good indicators for the effectiveness of antioxidants. Formation of high contents of dienes may be due to the higher content of polyunsaturated fatty acids, and also formation of conjugated trienes could be a result of dehydration of conjugate diene fatty acids [21]. On the whole, it can be stated that formation of hydroperoxides is a result of conjugation of double bonds and change in configuration of radicals present in fat such as peroxyl and alkoxyl radicals in polyunsaturated fatty acids, which can be measured by UV absorption [20].

Peroxide value (PV)

As can be seen (Table 3), PV in all the samples had an increasing trend due to open glass doors during heat and continuous peroxide formation in the presence of oxygen. During 5-25 min microwave heating, SHEO and ZMEO in concentration of 200 ppm showed better inhibitory effect on hydroperoxide production than control and other studied samples. Also, the PV of SHEO and ZMEO at concentration of 1000 ppm had no significant difference with control. Both of essential oils (200 and 1000 ppm) had, more or less, better antioxidant activities than BHT (200 ppm) during 20 min heating. Phenolic antioxidants have inhibitory effect against lipid peroxidation, while this effect is reduced over time due to decomposion and deterioration [21].

According to results in Table 3, during microwave heating (25 min), it is observed that the control sample over time has shown increased value; also, in treatments containing SHEO and ZMEO an increasing trend in peroxide value of each sample is observed. However, a good inhibitory effect was shown up to 15 min and finally an increasing trend was observed after this time with higher intensity, which is indicative of the instability of the essential oil and its effective components in higher heating intensity or longer period of exposure to heat. In general, the 200 ppm of SHEO showed the highest inhibitory effect on RBD soybean oil oxidation ($p < 0.05$).

Conjugated dienes and trienes

As can be seen (Tables 4 and 5) there is a direct correlation between heating time and CD and CT values. Due to open doors of experimental samples and the constant presence of oxygen and heat created by the microwave, peroxide value has been exponentially increased especially in last minutes.

As shown in Table 4, among the samples containing essential oil, 200 ppm SHEO had better inhibitory effect on conjugated diene formation in RBD soybean oil relative to other treatments; however, its effect has been inferior relative to 200 ppm BHT. Also, 200 ppm ZMEO had no significant difference with control, and 1000 ppm ZMEO had higher conjugate diene content than the control. On the other hand, Table 4 reveals that SHEO shows great resistance to the formation of diene conjugated like BHT and such effect is reduced for 1000 ppm concentration of SMEO after 15 min.

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Table 3 - Peroxide values (meq/kg) of heated soybean oil by microwave (600 W)

	Oil treatment					
Time (min)	**Control**	**BHT200 (ppm)**	**SHEO200 (ppm)**	**SHEO1000 (ppm)**	**ZMEO200 (ppm)**	**ZMEO1000 (ppm)**
5	1.7 ± 0.1^{c}	1.2 ± 0.2^{b}	1.4 ± 0.0^{bc}	1.2 ± 0.3^{b}	0.1 ± 0.0^{a}	1.3 ± 0.2^{bc}
10	3.1 ± 0.1^{c}	1.6 ± 0.4^{a}	1.5 ± 0.3^{a}	2.4 ± 0.4^{abc}	2.6 ± 1.0^{bc}	2.0 ± 0.4^{ab}
15	7.2 ± 0.6^{c}	6.3 ± 1.1^{c}	2.9 ± 0.3^{ab}	2.5 ± 0.2^{ab}	3.4 ± 0.3^{b}	2.0 ± 0.3^{a}
20	7.5 ± 0.1^{b}	8.1 ± 0.3^{b}	4.9 ± 0.3^{a}	8.2 ± 0.7^{c}	6.9 ± 0.7^{b}	13.5 ± 1.4^{d}
25	13.7 ± 1.1^{c}	8.5 ± 0.3^{a}	9.7 ± 0.8^{b}	14.2 ± 0.3^{c}	18.3 ± 0.3^{d}	14.1 ± 0.7^{c}

Values are the mean ± standard deviation (n =3). Values with different letters in each row are significantly different ($p < 0.05$)**.** Soybean oil containing with BHT (200 ppm), SHEO (200 and 1000 ppm) and ZMEO (200 and 1000 ppm), and control without any antioxidant

Table 4 - Absorbance at 232 nm of heated soybean oil (CD values) with microwave (600 W)

	Oil treatment					
Time (min)	**Control**	**BHT200 (ppm)**	**SHEO200 (ppm)**	**SHEO1000 (ppm)**	**ZMEO200 (ppm)**	**ZMEO1000 (ppm)**
5	5.4 ± 0.3^{c}	4.3 ± 0.3^{b}	4.3 ± 0.4^{b}	3.0 ± 0.5^{a}	6.0 ± 0.3^{cd}	6.1 ± 0.2^{d}
10	6.2 ± 0.5^{bc}	4.9 ± 1.1^{a}	5.4 ± 0.3^{ab}	5.5 ± 0.3^{ab}	5.9 ± 0.2^{bc}	6.7 ± 0.2^{c}
15	6.3 ± 0.4^{bc}	5.0 ± 0.3^{a}	5.6 ± 0.8^{ab}	5.5 ± 0.2^{a}	6.4 ± 0.2^{c}	6.9 ± 0.4^{c}
20	6.8 ± 0.4^{bc}	5.3 ± 0.2^{a}	6.6 ± 0.5^{bc}	6.3 ± 0.8^{b}	7.2 ± 0.2^{c}	7.5 ± 0.2^{c}
25	8.7 ± 0.2^{cd}	6.3 ± 0.4^{a}	6.8 ± 1.3^{ab}	7.6 ± 0.4^{bc}	7.9 ± 0.2^{cd}	9.0 ± 0.4^{d}

Values are the mean ± standard deviation (n =3). Values with different letters in each row are significantly different ($p < 0.05$)**.** Soybean oil containing with BHT (200 ppm), SHEO (200 and 1000 ppm) and ZMEO (200 and 1000 ppm), and control without any antioxidant

Table 5 - Absorbance at 270 nm of heated soybean oil (CT values) with microwave (600 W)

	Oil treatment					
Time (min)	**Control**	**BHT200 (ppm)**	**SHEO200 (ppm)**	**SHEO1000 (ppm)**	**ZMEO200 (ppm)**	**ZMEO1000 (ppm)**
5	1.8 ± 0.2^{a}	1.7 ± 0.2^{a}	1.7 ± 0.1^{a}	1.6 ± 0.2^{a}	2.0 ± 0.1^{b}	2.1 ± 0.2^{b}
10	2.3 ± 0.3^{a}	1.8 ± 0.6^{a}	2.1 ± 1.2^{a}	2.0 ± 0.7^{a}	2.1 ± 0.3^{a}	2.2 ± 0.2^{a}
15	2.8 ± 0.1^{c}	1.9 ± 0.3^{a}	2.1 ± 0.2^{ab}	2.5 ± 0.4^{abc}	2.7 ± 0.6^{bc}	2.6 ± 0.2^{bc}
20	2.8 ± 0.2^{b}	2.1 ± 0.2^{a}	2.4 ± 0.2^{ab}	2.7 ± 0.4^{b}	2.8 ± 0.5^{b}	2.7 ± 0.2^{b}
25	4.1 ± 0.3^{c}	2.5 ± 0.4^{a}	3.3 ± 0.3^{b}	4.6 ± 0.6^{c}	2.8 ± 0.2^{ab}	4.1 ± 0.2^{c}

Values are the mean ± standard deviation (n =3). Values with different letters in each row are significantly different ($p < 0.05$)**.** Soybean oil containing with BHT (200 ppm), SHEO (200 and 1000 ppm) and ZMEO (200 and 1000 ppm), and control without any antioxidant

Some physiochemical properties of microwave and oven heated (170°C for 120 min) sunflower and virgin olive oils have been measured, and a significant difference has been observed in parameters measured by UV-Vis absorption [14]. In another study, the effect of microwave heating on oxidative stability of corn oil has been evaluated by measuring PVs, acid value and UV absorption. Application of synthetic antioxidants did not show any inhibitory effect in microwave in high heat, unlike conventional oven heating, under similar conditions for the two treatments. Microwave energy causes greater changes in samples, and that is because of molecular friction present in this method [2].

According to the results of Table 5, the amount of conjugated trienes formed in RBD soybean oil under microwave heating conditions has been lower in 200 ppm SHEO and ZMEO than in other treatments; however, these two treatments have had less inhibitory effect than 200 ppm BHT. In addition, the

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Table 6 - Comparison of present results with some published works (during microwave heating)

Sample			Peroxide value		Conjugated diene		Conjugated triene		Power heating (Watt)	Heating time (min)	Reference
	Type of antioxidant	Concentration (ppm)	Control	BHT200[1]	Control	BHT200	Control	BHT200			
Soybean oil	*Satureja hortensis* L. essential oil	200	+	−	+	*	+	−	600 W	25	Present study
		1000	*	−	+	−	*	−			
	Zataria multiflora essential oil	200	−	−	*	−	+	*			
		1000	*	−	*	−	*	−			
Corn oil	BHT/BHA+ Citric Acid	100+200	+		−		*		800 W	36	[2]
	BHT/BHA	200	+		*		+				
	Citric Acid	100	+	+	*	*	*	−			
Canola oil	BHT/BHA+ Citric Acid	100+200	−		*		*		800 W	36	[23]
	BHT/BHA	200	*		*		*				
	Citric Acid	100	*	*	*	*	−	−			
Corn oil	Corncob extract	500	+	−	+	−	+	−	500 W	21	[22]
		1000	+	+	+	+	+	*			

+: Effective
*: Not effective
[1]Oil containing with BHT (200 ppm), and control without any antioxidant

amount of conjugated trienes of 1000 ppm SHEO and ZMEO had no significant difference with control. Results of Table 5 reveal that both of essential oils at the concentration of 200 ppm have great resistance to formation of triene conjugated while SHEO, in comparison with ZMEO, shows greater effect and which is similar to BHT.

According to the amounts of dienes and trienes formed, it seems that at higher essential oil concentrations their inhibitory effect decreases. This may be due to instability of these essential oils at high temperatures created in microwave conditions (160-180 ºC) or the conditions, which have led to prooxidant properties of essential oils used in this study under the condition of heating by microwave.

Conclusion

Despite the fact that plant essential oils are good sources of phenolic and antioxidant compounds especially phenolics, this study indicated that SHEO and ZMEO can reduce oxidation rate of soybean oil at high temperatures under microwave condition. Because of instability of hydroperoxides at high temperatures, it can be stated that analysis by UV absorption is a fast and appropriate method relative to peroxide value for measuring oxidation reaction in oils.

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