Antioxidant Activites of Thyme Oils Extracted from an Organic and a Chemical Treated Plants and their Influnce on Sunflower Oil Oxidation.

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ABSRACT

The essential oil of Thyme (*Thymis vulgaris* L.) is an aromatic member of the Lamiaceae family. Antioxidant and radical- scavenging properties of organic and chemical thyme oils were tested by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Results revealed that organic thyme oil a major effectiveness compared with chemical thyme oil. Efficient of organic and chemical thyme oil in stabilizing sunflower oil during accelerated storage has been studied. Organic and chemical thyme oils were prepared in different concentrations, peroxide value (PV), antioxidant effectiveness (AE), conjugated dienes (CD) and thiobarbituric acid number (TBA) were taken as parameters for evaluation of antioxidants effectiveness of organic and chemical thyme oils in stabilization of sunflower oil. Results from different parameters were in agreement with each other, suggesting the highest efficiency of SO+BHT 200 ppm, followed by SO+OTO 1000 ppm, SO+CTO 1200 ppm, SO+OTO 600 ppm, SO+CTO 600 ppm, SO+OTO 200 ppm, SO+CTO 200 ppm and Ctrl. Results revealed that Organic thyme oil has a potent antioxidant for stabilization of sunflower oil.

Key words: Organic thyme oil (OTO), Chemical thyme oil (CTO), Antioxidant activity, DPPH, Sunflower oil (SO) and Heating.

1. INTRODUCTION

Edible oils with higher contents of unsaturated fatty acids, especially polyunsaturated fatty acids, are more susceptible to oxidation. Lipid oxidation of oils not only can produce rancid odors, unpleasant flavors and discoloration, but can also decrease the nutritional quality and safety of an oil due to degradation products, resulting in harmful effects on human health (Esterbauer *et al.*, 1991; Lercker *et al.*, 2002). Due to the oxidation, consumers do not accept oxidized products while businesses suffer from economic loss (Maisuthisakul1 and Charuchongkolwongse, 2007). To retard or prevent the oxidative deterioration, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), terbutyl hydroquinone (TBHQ) have been widespread used as food additives in many countries but their safety has been questioned because recent reports revealed that these compounds may be implicated in many health risks, including cancer and carcinogenesis (Hou, 2003; Prior, 2004). Hence, there is a tendency towards the use of natural antioxidants of plant origin to replace these synthetic antioxidants.

Natural antioxidants are able to protect from free radicals and retard the progress of many chronic diseases and lipid oxidative rancidity in foods (Pryor, 1991; Kinsella *et al.*, 1993; Lai *et al.*, 2001; Gulcin *et al.*, 2003).

Thyme (*Thymus vulgaris* L.), locally known "zaatar" or "zaitra', a member of the family Lamiaceae, is an aromatic plant of the Mediterranean flora commonly used as spices and for medicinal purposes. Like other various Thymus species, thyme is traditionally used for its antiseptic, antispasmodic, and antitussive effects. Furthermore, thyme possesses antimicrobial, antifungal, antioxidative, and antiviral properties (Brasseur, 1983; Essawi and Srour, 2000; .Miura *et al.*, 2002; and Soliman and Badeaa, 2002). The essential oil derived from thyme (*T. vulgaris* L.) is a mixture of monoterpenes and one of the main compounds of this oil is a natural terpenoid thymol (Hudaib *et al.*, 2002). Thymol exhibits multiple biological activities including anti-inflammatory (Braga *et al.*, 2006), immunomodulating (Suzuki and Furuta , 1988), antioxidant (Aeschbach, *et al.*, 1994), antibacterial (Didry *et al.*, 1994 and Venturini *et al.*, 2002), antifungal (Mahmoud, 1994), and free radical scavenging properties (Fujisawa and Kadoma, 1992).

It has been reported that the essential oils extracted from thyme, and in particular the phenolic components (including carcavarol and thymol), were responsible for antioxidant activity observed in lipid systems (Farag *et al.*, 1989a; Deighton *et al.*, 1993 and Lee *et al.*, 2005). Hertrampf (2001) reported antibacterial, anticocidial, antifungal and antioxidant effects for thyme oil.

The important role of chemical fertilizers in increasing the medicinal and aromatic plants production is fully recognized. However, In recent years, many constraints have been raised due to their adverse impacts on public health, environment and national income. To control this problem, it is necessary to develop alternative methods of supplying nutrients to the growing plants. Many scientists consider the utilization of bio-organic fertilizers today as a promising altemative technique particularly for maintaining the fertility and productivity of agricultural soils, in addition to reducing the risk of environmental pollution (Nicholson *et al.*, 1999 and Galal and Ali, 2004).

Organic and Bio-fertilization technology has taken a part in minimizing production costs and avoiding the environmental hazards (Galal and Ali, 2004). The beneficial effect of organic and bio-fertilizer treatments on vegetative growth, yield, volatile oil and chemical composition of

Coriander plant were obtained by Abdalla (2009) and Surendra *et al.* (2002).

The aim of this work was to study the antioxidant activities of an organic and a chemical treated thyme oil and their influences on oxidation of sunflower oil during heating.

2. MATERIALS AND METHODS

2.1. MATERIALES:

2.1.1. An organic and a chemical treated thyme plant leaves (*Thymus vulgaris, L.*) were obtained from Syria. These plants were cultivated during season 2007/2008 in the area of Sheikh Bader (Tartous, Syria). The organic thyme plants were fertilized using an organic compost (sheep dung) at 30 ton/ha and its chemical composition were presented in Table (1). The Chemical treated thyme plants were fertilized using mineral fertilizers as follows: 450 kg ammonium sulfate/ ha, 400 kg superphosphate / ha and 150 kg / ha sulphate of potash.

Table (1): Chemical composition of organic fertilizer (sheep dung):

Micro elements, ppm			Relationships of organic matter dry weight		Macro elements, %		
Zn	Fe	Mn	O.M %	Ash %	N	P_2O_5	K_2O
32	6418	2.68	33.78	65.22	1.13	0.93	1.62

2.1.2. Chemicals:

All chemicals used (analytical grades) were purchased from El-Nasr pharmaceutical Chemical Company, Alexandria, Egypt. 2, 2 diphenyl-1-picryl hydrazyl (DPPH) and butylated hydroxtoulene (BHT) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Thiobarbituric acid (TBA) was obtained from Aldrich Chemical Co. Ltd., England). Sunflower oil was obtained from Arma Oils Co. 10^{th} of Ramadan, Egypt. Its peroxide value was 0.9 (meq O_2/kg oil).

2.2. METHODS:

2.2.1. Extraction of organic and chemical thyme oils: the thyme oil from both an organic and a chemical treated plant was obtained by steam distillation according to method described by (Raza *et al.*, 2009).

2.2.2. Antioxidant activity of an organic and a chemical treated thyme oil using (DPPH) method:

The scavenging ability on 2, 2 diphenyl-1- picryl hydrazyl (DPPH) radicals was determined according to the method of Shimada *et al.* (1992). The organic and chemical treated thyme oil (1.0- 10.0 mg/ml) in ethanol (4ml) was mixed with 1 ml of methanolic solution containing DPPH radicals, resulting in a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank. The scavenging ability was calculated as follow: Scavenging ability (%) = [(ΔA_{517} of control – ΔA_{517} of sample) / ΔA_{517} of control]. BHA was used for comparison.

2.2.3. Reaction mixture:

Different concentrations of organic thyme oil (200,600 and 1000 ppm) and chemical thyme oil (200, 600 and 1200 ppm) were added separately to the sunflower oil (100 g). The oxidation rates of sunflower oil were followed at 60 °C for 20 days.

2.2.4. Measurements of sunflower oil oxidation:

2.2.4.1. Peroxide value (PV):

Peroxide value (PV) of the oil was determined according to AOCS official methods (1989) (Method Cd8-53) by titration with standard sodium thiosulphate (0.1 N) and was calculated as mil equivalent peroxides per kilogram oil (meqO_2/kg oil).

2.2.4.2. Antioxidant Effectiveness (AE):

The percentage antioxidant effectiveness (AE) was calculated from the equation reported by Adegoke and Gopala Krishna (1998):
AE % = (PV of control – PV of test sample / PV of control) * 100.

2.2.4.3. Conjugated diene (CD):

Absorption at 232 nm was evaluated using spectrophotometer (Safas Monaco, 1900), using a 1% solution of oil in cyclohexane and a path length of a cm. The absorption at the specified wavelength (232 nm) in the method is due to the presence of conjugated diene system (Abdalla and Roozen, 1999).

2.2.4.4. Thiobarbituric acid number (TBA) of oils:

TBA number of the oil was determined according to Allen and Hamilton (1989). The resultant solutions were measured at 538 nm using spectrophotometer (Safas Monaco, 1900). The TBA number was calculated from the following equation:

TBA number = 7.8 * D mg malonaldehyde per kg oil

Where: D is the absorbency against blank at 538 nm.

2.2.5. Sensitivity (threshold) test:

This test was carried out according to Farag *et al.* (1989b).Two sets of beakers (8 each) containing sunflower oil were mixed with different concentrations of organic and chemical thyme oils (100, 200, 400, 600, 1000, 1200, 2000 and 3000 ppm). Panelists (8) were chosen from personnel within the Faculty of Agriculture, Saba Basha, Alexandria University, and asked to sniff each sample, characterized the odors and rate the odor intensity on a scale 0 (no odor) to 5 (extremely strong odor).

3. RESULTS AND DISCUSSION

3.1. Antioxidant activity of organic and chemical thyme oils on free radical scavenging (DPPH).

Scavenging of DPPH free radicals is a widely used method to evaluate antioxidant activity of specific compounds or extracts in a relatively short time. Because of the easy and convenience of this reaction, it now has widespread use in free radicals scavenging assessment (Thaipong *et al.*, 2006, Siddhuraju, 2007 and Lee *et al.*,2007). The reduction in the DPPH radical absorption at 517 nm by the action of antioxidants is taken as a measure of antioxidant activity (Ancerewicz *et al.*, 1998).

Data of Table (2) showed that the organic thyme oil had higher scavenging activities on DPPH radicals than the chemical thyme oil. However, the scavenging effect of BHT at 10 mg/ml was 96.7%. These results indicated that that organic thyme oil was higher in antioxidants and phenols. Abdl Aziz, and Hussein (2010) concluded that organic fertilizer gave higher percentage of essential oil and more of major compounds percentage (especially, Carvacrol, Thymol and p-cymene) than chemical fertilizer.

Table (2): Scavenging activity of organic and chemical thyme oil on 2, 2 diphenyl-1- picryl hydrazyl radical (DPPH) compared to that of butylated hydroxytoulene (BHT).

Concentration (mg/ml)	Scavenging Activity (%)		
	Organic Thyme Oil	Chemical Thyme Oil	BHT
1	17.5	16.2	20.1
2	23.9	22.7	27.8
3	29.3	27.9	35.1
4	34.4	32.3	42.3
5	42.7	40.7	50.8
6	50.2	48.1	58.1
7	58.6	57.1	65.2
8	69.3	68.2	75.1
9	78.9	76.4	85.2
10	89.7	85.2	96.7

3.2. Antioxidant activities of organic and chemical thyme oils in sunflower oil during heating.

Organic and chemical thyme oils were tested for their antioxidants activities under accelerated storage of sunflower oil as oxidation substrate. The oxidative deterioration level was monitored for the measurements of peroxide value, antioxidant effectiveness, conjugated diene and TBA test. Also, the acceptability to mankind of organic and chemical thyme oils when mixed with sunflower oil was evaluated.

In this study, the addition of organic and chemical thyme oils at various concentrations did not affect the color or the appearance of sunflower oil at all. An experiment was conducted where sunflower oil was catalyzed by BHT (200 ppm) (Food grade) in order to compare the antioxidant efficiency of the organic and chemical thyme oils with the most commonly used synthetic antioxidant material.

Organic and chemical thyme oils at 1000, 1200 ppm, respectively produced an antioxidant power near and similar to that produced by BHT at

200 ppm. The level of organic and chemical thyme oils were 5 and 6 times, respectively to that of BHT. However, natural antioxidants are preferred over synthetic antioxidants, to minimize adverse effect on mankind. These results are agreed with those obtained by Farag *et al.*, (1989b).

3.2.1. Peroxide Value (PV):

The peroxide value, which measure hydro peroxide products of the oils is a good indicator of the oxidation state of the oils (Mc Ginely, 1991).Peroxide value (PV) of sunflower oil with different concentrations of organic and chemical thyme oils as well as the effect of heating at 60 °C for 20 days were investigated and are shown in Figures (1 and 2). Results revealed that, the control had the highest PV (60.1 meqO_2/kg oil) after heating at 60 °C for 20 days. On the other hand, sunflower oil with 200 ppm BHT had the lowest PV (30.6 meqO_2/kg oil). Data also showed that PV of sunflower oil with different concentrations of organic thyme oil was lower than those of chemical thyme oil during heating at 60 °C for 20 days. The higher the concentrations of thyme oils, the lower the PV were.

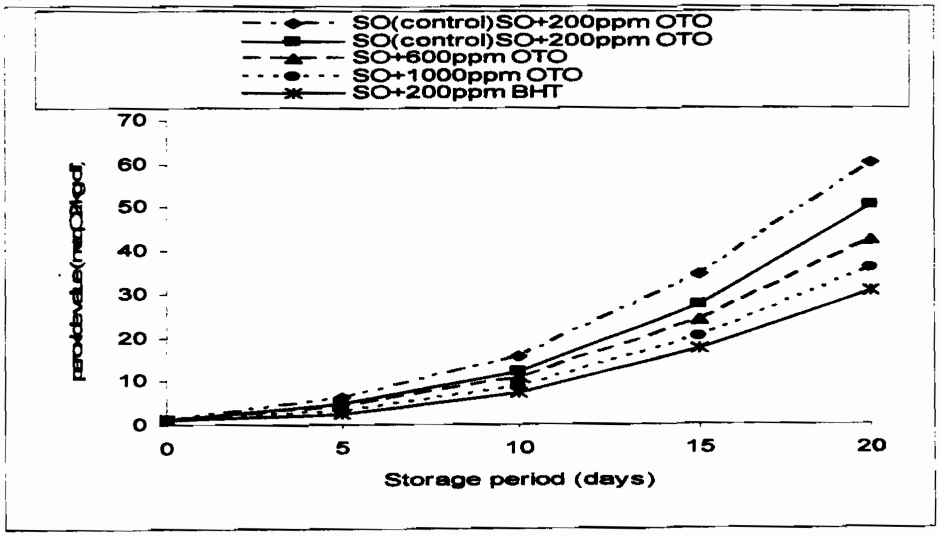

Figure (1): Peroxide value of sunflower oil (SO) without and with added different concentrations of organic thyme oil (OTO) during storage at 60 °C for 20 days.

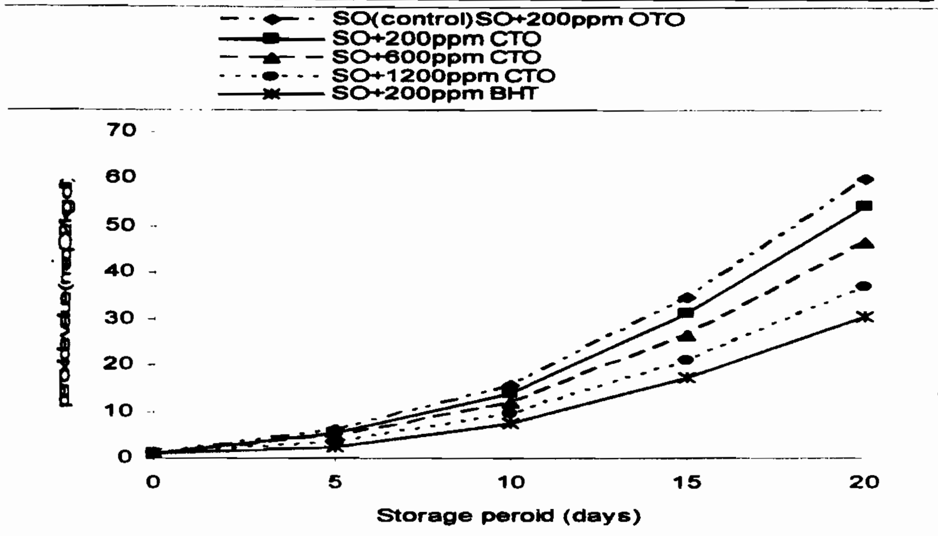

Figure (2): Peroxide value of sunflower oil (SO) without and with added different concentrations of chemical thyme oil (CTO) during storage at 60 °C for 20 days.

3.2.2. Antioxidant effectiveness (AE):

Table (3) shows the antioxidant effectiveness percentages of organic and chemical thyme oils in sunflower oil during heating at 60 °C for 20 days. Data revealed that, addition of organic thyme oil at concentrations 200, 600, 1000 ppm and chemical thyme oil at concentrations 200, 600, 1200 ppm to sunflower oil showed antioxidative effect as compared to a synthetic antioxidant BHT. The antioxidative effect of organic and chemical thyme oils increased as the concentration of organic and chemical thyme oils added to sunflower oil increased. Data also revealed that the antioxidant effectiveness of organic thyme oil was higher than those of chemical thyme oil added to sunflower oil. Similar results were found for used black tea (Barbary, 2000).

Table (3): The antioxidative effectiveness (AE) % of different concentrations of organic and chemical thyme oil on sunflower oil during storage at 60 °C for 20 days.

Storage time (Days)	Antioxidative Effectiveness (AE)[a] %							
	Organic Thyme Oil				Chemical Thyme Oil			
	200 ppm	600 ppm	1000 ppm	BHT 200 ppm	200 ppm	600 ppm	1200 ppm	BHT 200 ppm
5	22.58	32.26	50.0	59.68	11.29	25.81	43.55	59.68
10	21.29	30.97	43.87	52.26	10.97	23.23	39.35	52.26
15	20.0	30.14	41.45	49.86	9.86	23.19	38.55	49.86
20	15.97	29.28	40.60	49.08	9.82	22.46	38.27	49.08

(a) **AE % = (PV of control – PV of test sample / PV of control) * 100.**

3.2.3. Conjugated diene (CD):

The specific extinctions, in term of conjugated diene (CD) at 232 nm are considered important parameter for the investigation of primary oxidative deterioration of the oils (Yoon *et al.*, 1985) and thus a good indicator of the antioxidant effectiveness of the antioxidants of organic and chemical thyme oils. Figure (3 and 4) showed the increase in the content of conjugated diene (CD) during storage time for 20 days at 60 ºC. The highest increase in CD was observed for control as compared to those other concentrations of thyme oil. Formation of CD was lower in sunflower oil with organic thyme oil than those of chemical thyme oil added to sunflower oil at different concentrations. Organic thyme oil was more effective in retarding formation CD and higher antioxidant than chemical thyme oil.

Peroxide value and conjugated diene at 232 nm showed the same tendency, indicating a very strong correlation between these two methods (Almeida-Doria and Regitano-D'arce, 2000 and Anwar *et al.*, 2006).

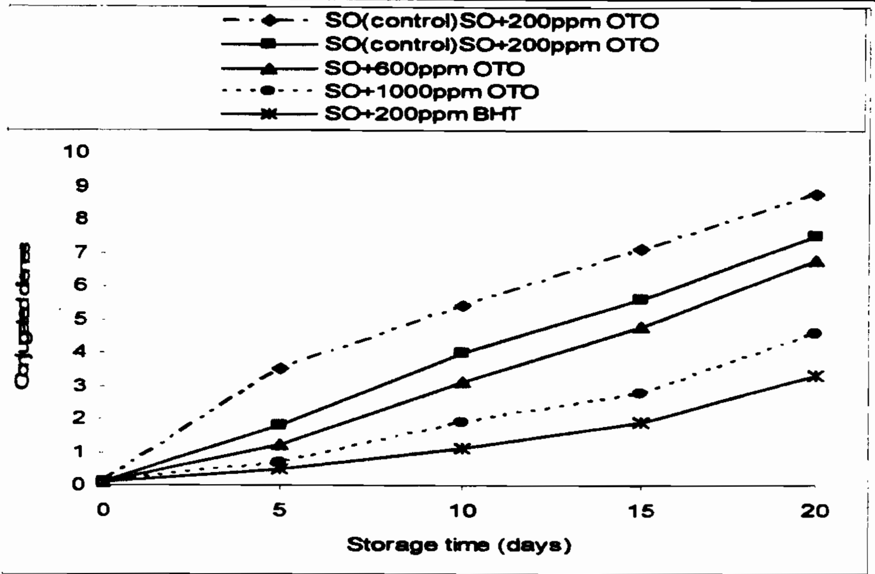

Figure (3): Conjugated diene (CD) of sunflower oil (SO) without and with added different concentrations of organic thyme oil (OTO) during storage at 60 °C for 20 days.

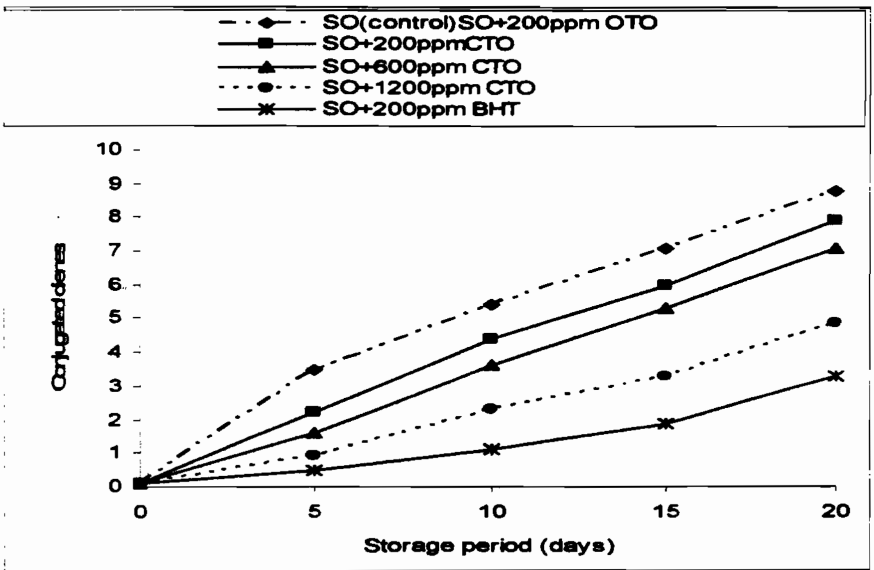

Figure (4): Conjugated diene (CD) of sunflower oil (SO) without and with added different concentrations of chemical thyme oil (CTO) during storage at 60 °C for 20 days.

3.2.4. Thiobarbituric acid (TBA) test:

Effect of different concentrations of organic and chemical thyme oil on the TBA values of sunflower oil during heating at 60 °C for 20 days is given in Figures (5 and 6). The TBA test measures a secondary product of lipid oxidation, malonaldehyde. It was assumed that accumulation of these products during consecutive days of storage affected the oil quality and was responsible for the development of rancid odor and off-flavor of the oil. Results, revealed, again, the same trend as for PV and CD. The TBA values of sunflower oil with different concentrations of organic and chemical thyme oil increased with heating time. Control sample had the highest TBA values, while sunflower oil with BHT (2oo ppm) and sunflower oil with organic thyme oil (1000 ppm) gave the lowest TBA values, respectively. TBA values of sunflower oil with different concentrations of organic thyme oil were lower than those of chemical thyme oil.

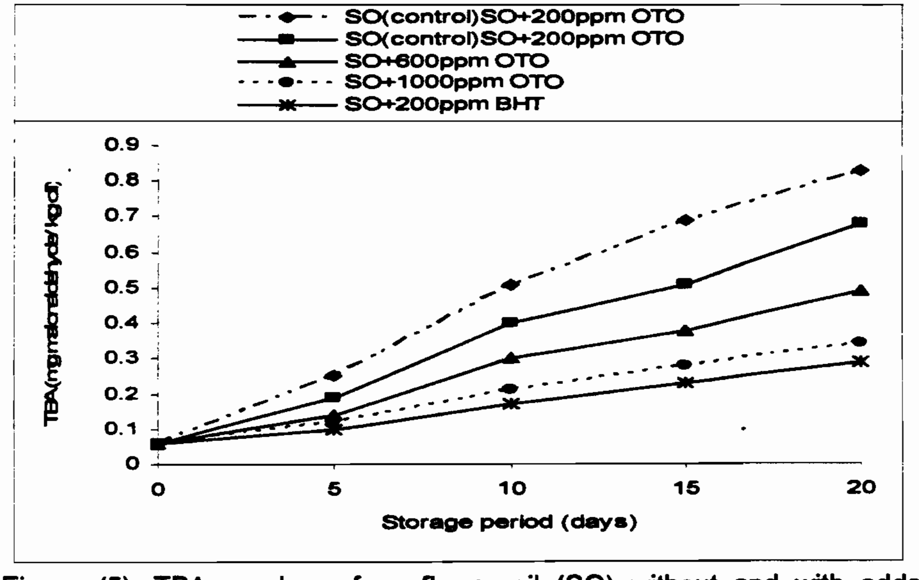

Figure (5): TBA number of sunflower oil (SO) without and with added different concentrations of organic thyme oil (OTO) during storage at 60 °C for 20 days.

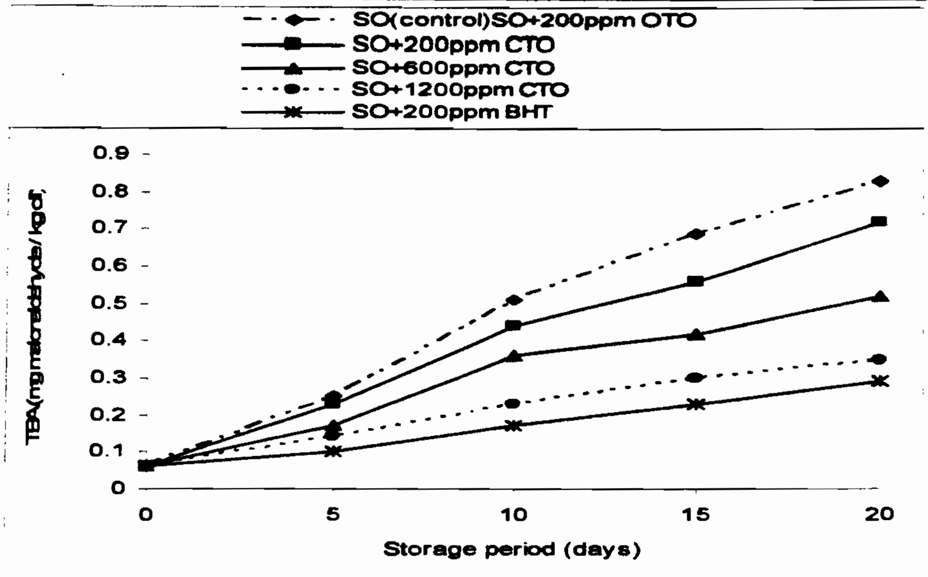

Figure (6): TBA number of sunflower oil (SO) without and with added different concentrations of chemical thyme oil (CTO) during storage at 60 °C for 20 days.

3.3. Threshold test:

A set of experiments was conducted to detect the acceptability and threshold of sunflower oil mixed with organic and chemical thyme oils. The threshold values for these systems are shown in Table (4). These values showed that sunflower oil mixed with organic and chemical thyme oils were the same at the range of 100 to 600ppm did not at all affect the odor note of sunflower oil and consequently the mixed sunflower oil is completely acceptable for human consumption. The addition of organic thyme oil to sunflower oil at 1000, 1200, 2000, 3000 ppm possessed weak, medium, medium and strong odor, respectively. This organic thyme oil should be added below concentration 1000 ppm. However, chemical thyme oil mixed with sunflower oil at 1000, 1200, 2000, 3000 ppm caused none, weak, medium and strong odor, respectively. Chemical thyme oil should be added below concentration 1200 ppm. Similar results obtained by Farag *et al.* (1989b).

Table (4): Mean threshold values for organic and chemical thyme oil added to sunflower oil.

Concentration (ppm)	Organic Thyme Oil		Chemical Thyme Oil	
	Detection of difference	Odor score [b]	Detection of difference	Odor score [b]
100	None	0.0	None	0.0
200	None	0.0	None	0.0
400	None	0.0	None	0.0
600	None	0.1	None	0.0
1000	Weak	1	None	0.0
1200	Medium [a]	1.9	Weak	0.9
2000	Medium	2.4	Medium [a]	2.0
3000	Strong	3.3	Strong	3.0

[a] Threshold value refer to the minimum concentration at which a stimulus is easily characterized.

[b] The intensity of odor was described according to the following scale :0, None (odor of control); 1, weak (odor different from control); 2, medium; 3, strong; 4, very strong; 5, extremely strong.

Finally, to conclude, organic thyme oil was more than chemical thyme oil in antioxidant activity, antioxidant effectiveness and free radical scavenging.

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الملخص العربى

النشاط المضاد للأكسدة لزيوت الزعتر المستخلص من نباتات معامله عضويا و كيميائيا و تأثيرها على اكسدة زيت زهره الشمس .

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يعد زيت الزعتر من الزيوت العطريه الهامة و التى لها خواص مضادة للاكسدة فتم دراسه نشاط مضادات الأكسدة لزيت الزعتر العضوى و الكيماوى من خلال قدره و كفاءة زيت الزعتر على خلب العناصر الشره الحره free radicals scavenging و اوضحت النتائج ان كفاءه زيت الزعتر

العضوى على خلب العناصر الشرة الحرة اعلى من كفاءة زيت الزعتر الكيماوى . و عند اضـــافه زيـــت الزعتر العضوى و الكيماوى على زيت زهره الشمس اثناء التسخين على درجه حرارة 60 °م لمـــدة 20 يوم و بتركيزات مختلفه و مقارنتهم بمضاد اكسده صناعى BHT وجد ان رقـــم البيروكـــسيد و التـــأثير المضاد للاكسدة و الأحماض المترافقة و رقم TBA للزيت يزداد مع زياده التسخين و اعطى BHT اقل زياده ثم زيت الزعتر العضوى بتركيز 1000جزء فى المليون ثم زيت الزعتر الكيماوى بتركيز 1200 جزء فى المليون. و اوضحت النتائج ان كلما زادت التركيزات كلما زاد كفاءه زيـــت الزعتـــر كمـــضاد للاكسدة و ان اقصى تركيز يمكن اضافته إلى زيت زهره الشمس من زيت الزعتر العضوى و الكيمـــاوى و لا يستطيع الانسان تميزه هو أقل من 1000 ، 1200 جزء فى المليون على الترتيب.