Effects of some Aromatic Plant Oils on Thermo Oxidative Stability of Sun Flower Oil

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Abstract:

Essential oils were obtained from four selected aromatic plants namely: clove, fennel, rosemary and thyme. Their contents of total polyphenols were determined. The antioxidant activities of the studied aromatic plant oils on sunflower oil comparing with the synthetic antioxidant Butylated Hydroxy Toluene (BHT) were determined by a Rancimat apparatus. Antioxidative effect of the extracted aromatic plant oils on the oxidative stability of sunflower oil during heating up to 18 hours was also evaluated. The obtained results indicated that, the studied aromatic plants were rich in phenolic contents which ranged from 9.32 to 19.82 mg as gallic acid/100g on dry weight base.

The highest level of total phenolics was found in rosemary followed by fennel and clove, while thyme recorded the lowest.

The induction periods determined by Rancimat for sunflower oil treated by 200 ppm of (BHT) or 400 ppm of extracted aromatic plant oils were greater than that of control with more efficiency by using the aromatic oils. Beside, addition of aromatic plant oils to sunflower oil minimized peroxidation and increased its oxidative stability during hating up to 18 hours.

Keywords: aromatic plant, polyphenols, antioxidant, induction period, sunflower oil.

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Introduction:

Oxidative degradation of lipids is a major factor limiting the shelf life of foods. The free-radical reaction of lipid peroxidation is generally responsible for the deterioration of lipid-containing foods.

Lipid oxidation decreases the nutritional and sensory properties of foods since it involves the loss of essential fatty acids and vitamins and generation of toxic compounds, causing additionally, flavor, texture and color deterioration (Morrissey *et al.*, 1998).

Use of antioxidants during the manufacturing process can minimize the extent of lipid peroxidation (Shahidi and Wanasundara, 1992).

There is increasing interest in the industry and scientific research for spices and aromatic plants because of their strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants. These properties are due to many substances, including some vitamins, flavonoids, terpenoids, carotenoids, etc. and render spices and some aromatic plants or their antioxidant components are use as preservative agents in food (Calucci *et al.*, 2003).

Polyphenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity (Kahkonen *et al.*, 1999). Aromatic plants are used in many domains, including nutrition, flavoring and beverages (Djeridane *et al.*, 2006).

Many aromatic plants have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, antimicrobial, hypolipidemic and anticarcinogenic potential (Luo *et al.*, 2004).

Crude extracts of aromatic plants and other plant materials rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food.

Deep fat frying was an important processing procedure used worldwide for the preparation and production of food. During the frying process, the frying fat was heated or reheated over an extent period of time and quality changes occurred in the frying fat that might adversely affect the flavor and nutritional value of foods (Smith *et al.*, 1986). Thus, maintaining quality of fats and oils used for deep frying was important in food preparation.

Kochhar (2000) stated that, the natural way of improving oxidative and flavor stability of frying oils and fats is by adding natural antioxidative components and precursors present in the plant kingdom such as aromatic plant oils.

The present work was carried out in an attempt to evaluate the utilization of some aromatic plant oils as a source of natural antioxidants. Antioxidative effect of isolated natural antioxidant on thermo oxidative stability of sunflower oil during heating at 180°C ± 5°C up to 18 hours was evaluated as well.

Materials and Methods:

1. Materials:

Four selected aromatic plants, namely: Clove (*Syzygium aromaticum*), fennel (*Foeniculum vulgare*), rosemary *(Rosmarinus officinalis)* and thyme (*Thymus vulgaris L.)* were obtained from a private farm in Assiut governorate.

Refined sunflower oil free from antioxidants was donated from El-Nile Company for Oils and Detergents, Assuit, Egypt. Butylated Hydroxy Toluene (BHT) was purchased from Sigma, Chemical Company (St. Louis, USA).

The initial characteristics of sunflower oil used in this study were checked by determining acid, peroxide, iodine and conjugated diene and triene values as described below.

The food additive regulation in USA had a limitation of 200 ppm of synthetic antioxidant, while others of natural antioxidants had 400 ppm as a recommended usage level (Eastman Chemical Company, 1993).

2. Methods:

2.1. Extraction of essential oils:

The essential oils of fennel seeds, rosemary leaves, thyme leaves and clove buds were obtained by water distillation according to the method described in the British Pharmacopeia (1988) by using Clevenger apparatus. The volatile oil was collected after 4 hours of distillation (until no more essential oil was obtained). The separated volatile oil was dried over anhydrous sodium sulphate before hold in glass bottles at -18°C until analysis.

2.2. Total Polyphenols extraction:

Distilled plant material was dried in a forced-air drier at 35°C for 48h (until it reached a constant weight) and then ground to pass a 2-mm sieve. Dried samples (0.5 g) were firstly extracted with 20mL of petroleum ether under stirring and taken to dryness at room temperature. Secondly, they were extracted using 150 mL of methanol in a Soxhlet extractor (B-811) (Buchi, Flawil Switzerland), for 2h under a nitrogen atmosphere. Methanolic extracts were taken to dryness at 40°C under vacuum conditions in an evaporator system (Syncore Polyvap R-96) (Buchi, Flawil, Switzerland). The residue was re-dissolved in methanol and made up to 5mL. The concentration of the extracts was expressed in terms of dry weight per mL of solvent. The extracts were kept in vials at −80°C until their corresponding analysis.

2.3. Total polyphenols content:

Total polyphenol content was demonstrated using Folin-Ciocalteu colorimetric method as described by Huang *et al*. (2007).

The absorbance of the resulting blue color was measured at 765 nm with a Shimadzu UV spectrophotometer. Quantification was done with respect to the standard curve of gallic acid. The results were expressed as mg gallic acid/100 g dry weight.

2.4. Antioxidant activity:

Antioxidant activities of the studied aromatic plant oils compared with synthetic antioxidant (BHT) were determined with a Rancimat apparatus (Metrohm, Herisau, Switzerland) by measuring the induction period of oils containing the antioxidant, according to the method described by Hasenhuettl and Wan (1992).

- The antioxidant was calculated as:

Antioxidant index = Induction period of oil with extract / Induction period of oil alone.

2.5. Thermal treatment of oils:

The two antioxidants (synthetic and natural extracted) used were within the recommended usage levels as previously mentioned.

Antioxidants were dissolved in 250 ml sunflower oil and kept in brown glass bottles for the following:

A. The sunflower oil samples were heated at 180°C ± 5°C for 18 hours at intervals of 3 hours heating for 6 consecutive days. The heated oils were sampled every day after heating in brown bottles and kept at 5°C for analytical experiments. The remainder oil samples were heated the next day for another 3 hours and then sampled as previously until the sixth day and the total of 18 hours heating.

B. The heated oil samples without adding antioxidant (control) and with the added antioxidants were used for determination of acid, peroxide and iodine values, conjugated diene and triene to follow the effect of the added antioxidants at high temperature compared to the control.

2.6. Physical and chemical properties of oils:

Acid value, iodine value, peroxide value, conjugated diene and triene were estimated according to AOCS (1998).

Results and Discussion:

1. Total phenolic contents:

The amount of total phenolic contents estimated by Folin-Ciocaltue method in the studied aromatic plant samples are presented in Table (1).

Tabulated data showed that, the amount of total phenolics varied widely in the studied selected aromatic plants, and ranged from 9.32 to 19.82 mg gallic acid/100 g of dry weight.

Table (1): Total phenolic content (mg gallic acid / 100g dry weight) in aromatic plants.

Aromatic plant	Total phenolic content
Clove	15.82
Fennel	17.60
Rosemary	19.82
Thyme	9.32

The highest level of total phenolics was found in rosemary, while the lowest was in thyme. The obtained results in the present study showed that the spices were relatively high in polyphenols. Total polyphenolic contents of the studied spices were decreased in the following order: rosemary > fennel > clove > thyme. Clear differences between the results were likely due to genotypic and environmental differences within species, choice of parts tested, and time of taking samples (kim and Lee, 2004; Shan *et al*., 2005).

2. Antioxidant activity of aromatic plant oils:

Antioxidant activities of studied aromatic plant extracts are presented in Table (2).

Table (2): Effect of aromatic plant oils on sunflower oil oxidative rancidity:

Sample system	Induction period (hr)	Antioxidant activity
Sunflower oil (Control)	7.60	-
Sunflower oil + Clove oil	15.95	2.10
Sunflower oil + Fennel oil	18.20	2.40
Sunflower oil + Rosemary oil	20.37	2.68
Sunflower oil +Thyme oil	15.23	2.00
Sunflower oil + BHT	13.10	1.72

The antioxidant activities of the essential oils extracted from clove buds, fennel seeds, rosemary and thyme were assessed by the Rancimat instrument. This method assigned the induction period for the onset of oxidative rancidity in sunflower oil at 100°C and the longer induction period indicates the stronger antioxidant activity.In the present study, simple model system comprise sunflower oil treated by one of the essential oils was designated to assess its oxidation behavior. An experiment was performed where sunflower oil was treated by BHT (200 ppm) in order to compare the antioxidant efficiency of the essential oils under study with the most commonly used synthetic antioxidant materials.

Table (2) show the effect of essential oils of clove bud, fennel seeds, rosemary and thyme on the oxidative rancidity of sunflower oil. Sunflower oil without any essential oil and sunflower oil mixed with BHT (200 ppm) were used as a guide to indicate the antioxidant phenomenon and also to compare the antioxidant power possessed by different natural antioxidants. Consequently, the results illustrated that all studied essential oils exhibited antioxidant activity. Moreover, the induction periods for all studied systems were greater than that of control (sunflower oil without additives). This means that all the added essential oils possessed antioxidant effect. From the abovementioned results it could be concluded that the studied aromatic plant extracts contain phenolic compounds and were capable of minimizing oil peroxidation, protecting sunflower oil against autoxidation (Arouma *et al*., 1992). These results are in good agreement with those reported by Sanhueza *et al*. (2000); Zheng and Wang (2001) and Pizzale *et al*. (2002). On the other hand, the induction periods as well as antioxidant activity of the treated sunflower oils by the studied aromatic oils reflected their contents of total phenolics as antioxidant which indicated in Table (1).

3.Antioxidative effect of aromatic plant oils on the stability of sunflower oil during heat treatments:

The refined vegetable oil selected for the study was sunflower oil. The selection of this oil was made owing to its common use as frying oil and also high relative reaction rates of its unsaturated fatty acids with oxygen (List and Erickson, 1985). To follow the oxidation rate in oil samples during heating up to 18 hours, the samples were analyzed periodically for acid value, iodine value, peroxide value, conjugated diene and conjugated triene values, since a single reaction criterion is not enough to account for the oxidative changes at various stages of heating.

The tabulated data in Table (3) and illustrated in Fig. (1) show the effect of heating time on the acid value of sunflower oil treated with extracted aromatic plant oils. Acid value reflected the degree of oil hydrolysis and the amount of free fatty acids involved in the heating of oil samples. The tabulated data revealed that, heating of sunflower oil caused an increase in the acid value. Such increment could be attributed to formation of acidic compounds and free fatty acids. Increases in free fatty acids were seen for all the heated oils at prolonged heating times, with the same trend also reported in other studies (Che Man and Jaswir, 2000 and Xu *et al*., 1999).

The great increases in acid value of the control heated oil (Table 3) which confirms that the unprotected heating oil is more susceptible to degradation during heating than the heating oils with the addition of antioxidants. At the end of heating periods, the acid value of the control, BHT, rosemary, fennel, clove and thyme extracted oils were 2.16, 1.90, 1.48, 1.60, 1.75 and 1.80%; respectively. Thus as shown in Fig. (1), the aromatic plant oils extract lowered free fatty acid formation during heating, has also been reported in other studies (Che Man and Jaswir, 2000 and Che Man and Tan, 1999).

Table (3): Effect of aromatic plant oils on the acid value (% of oleic acid) of sunflower oil during heating periods.

Heating periods (hours)	Sunflower oil samples					
	Control	Oil + BHT	Oil + Rosemary	Oil + Fennel	Oil + Clove	Oil + Thyme
Zero	0.38	0.38	0.38	0.38	0.38	0.38
3	0.90	0.72	0.50	0.61	0.62	0.66
6	1.22	0.96	0.72	.80	.85	0.90
9	1.46	1.20	0.96	.98	1.05	1.10
12	1.68	1.42	1.15	1.24	1.30	1.38
15	1.84	1.64	1.36	1.50	1.52	1.60
18	2.16	1.90	1.48	1.60	1.75	1.80

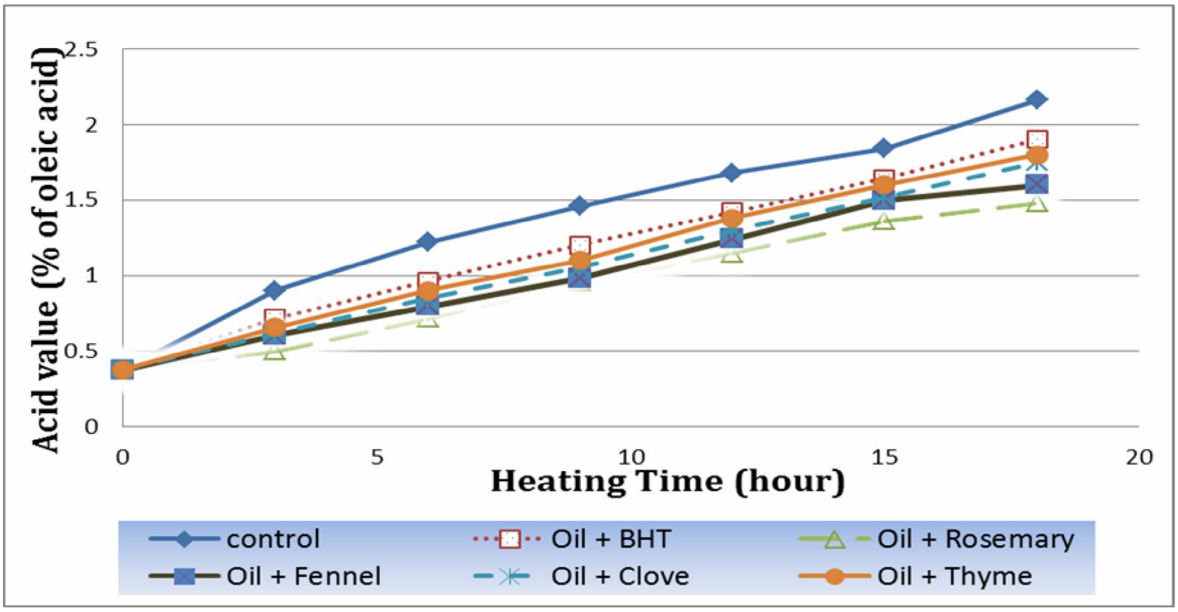

Fig. (1): Effect of aromatic plant oils on the acid value (% of oleic acid) of sunflower oil during heating periods.

This indicates that the natural antioxidants in the aromatic plant oils extract protected the sunflower oil from hydrolysis, as also seen for BHT but by more efficiency.

During heat treatment, a progressive decrease in unsaturation was observed in all studied samples by measurement of iodine value (Table 4 and Fig. 2). This decrement could be attributed to the destruction of double bonds by oxidation, scission and polymerization (Cuesta *et al*., 1991). Tabulated data showed that heating of oil substantially reduced the iodine values. Oxidation, which consisted of a complex series of chemical reactions, was characterized by a decrease in the total unsaturated content of the oil due to abstraction of hydrogen adjacent to double bond and the formation of free radicals. Hence heating that accelerated the oxidation of the oil caused maximal reduction of the iodine values (Gertz *et al*., 2000 and Paz and Molero, 2001).

The effect of adding antioxidants (BHT, clove, fennel, rosemary and thyme oils) on iodine value of the sunflower oil was analyzed after heating up to 18 hours. All antioxidants effectively reduced the oxidation rate in the oil, as detected by the values of iodine comparing with control samples (Fig. 2). Such results are in good accord with those reported by Naz *et al*., (2004) and Naz *et al*., (2005).

Table (4): Effect of aromatic plant oils on the iodine values (g I_2/100g oil) of sunflower oil during heating periods.

Heating periods (hours)	Sunflower oil samples					
	Control	Oil + BHT	Oil + Rosemary	Oil + Fennel	Oil + Clove	Oil + Thyme
Zero	127.62	127.62	127.62	127.62	127.62	127.62
3	124.80	125.40	126.82	126.60	125.86	125.62
6	123.20	125.13	125.72	125.50	125.40	125.20
9	121.68	123.90	124.40	124.10	123.82	123.76
12	119.40	121.80	122.90	122.20	122.00	121.96
15	118.96	120.90	121.30	121.00	120.80	120.72
18	116.70	118.80	120.20	119.60	119.20	119.00

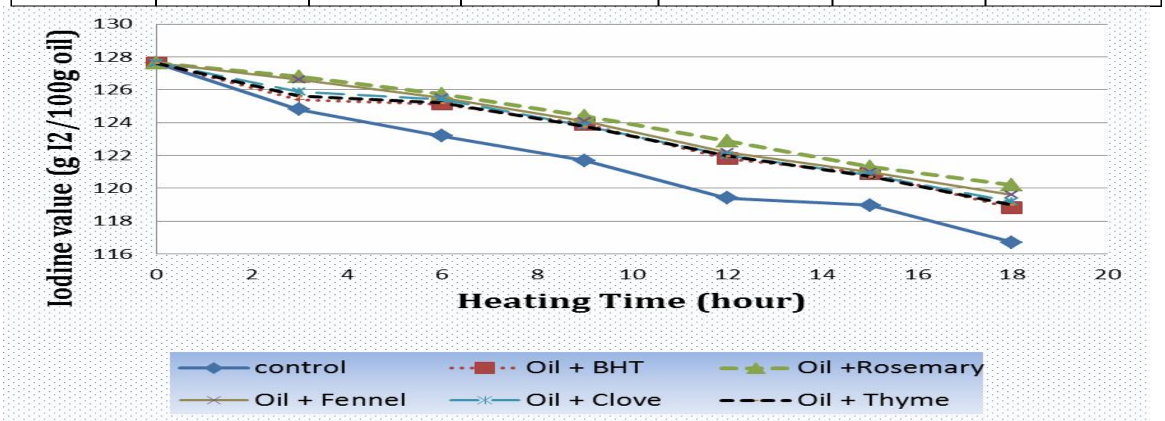

Fig. (2): Effect of aromatic plant oils on the iodine value (g I_2/100g oil) of sunflower oil during heating periods.

Peroxide formation in sunflower oil during heating up to 18 hours is shown in Table (5) and Fig. (3). The peroxide values were increased with incremented heating time up to 12 hours and then decreased. This may be due to the difference rate between peroxide formation and its decomposition. At the beginning of heating, peroxide formation was faster than its decomposition. However, the decomposition was the case as the heating proceeded. The effects of adding antioxidants (BHT and extracted aromatic plant oils) on peroxide value of the sunflower oil were tested after heating up to 18 hours. The results revealed that all studied antioxidants effectively reduced the oxidation rate in the oil, as detected by law values of peroxide comparing to sunflower oil without antioxidant (Fig. 3). These results are in agreement with those reported by Lee *et al*., (1994).

Table (5): Effect of aromatic plant oils on the peroxide values (meq / kg oil) of sunflower oil during heating periods.

Heating periods (hours)	Sunflower oil samples					
	Control	Oil + BHT	Oil + Rosemary	Oil + Fennel	Oil + Clove	Oil + Thyme
Zero	2.62	2.62	2.62	2.62	2.62	2.62
3	4.40	3.89	3.40	3.56	3.64	3.80
6	7.86	5.60	4.20	4.82	5.00	5.20
9	9.34	8.97	6.30	6.86	7.20	7.62
12	10.83	9.64	7.80	7.96	8.40	8.86
15	6.38	8.82	7.40	7.20	7.64	7.50
18	5.20	7.20	6.82	6.50	6.25	6.10

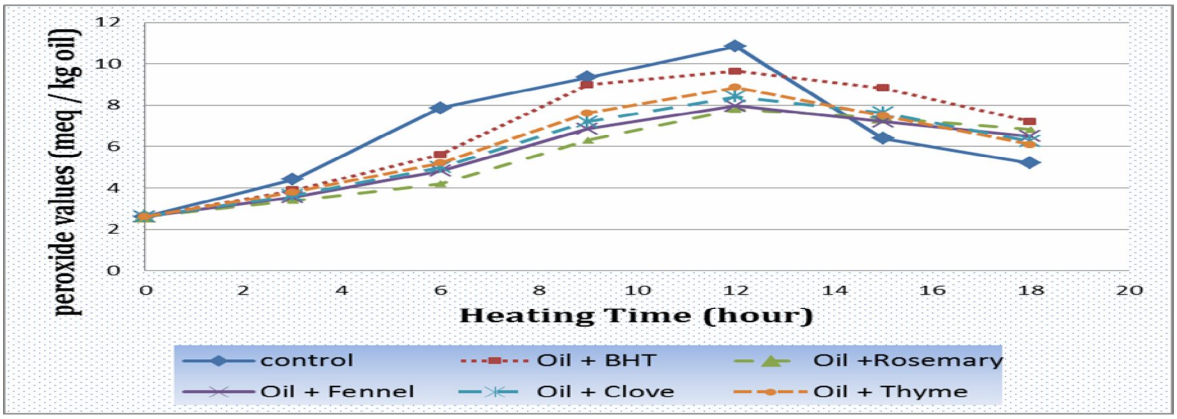

Fig. (3): Effect of aromatic plant oils on the peroxide value (meq / kg oil) of sunflower oil during heating periods.

Oils containing polyunsaturated fatty acids are oxidized to conjugated diene and triene systems, that could be measured by ultraviolet absorption at 232 nm and 268 nm; respectively (Gray, 1978).The oxidation of polyunsaturated fatty acids was accompanied by an increase in the ultraviolet (UV) absorbance with a maximum at about 232 nm, which was characteristic of conjugated diene systems (Table 6 and Fig. 4).Absorbance at 232 nm provides a measure of the content of conjugated dienes, which represent the degree of production of the primary oxidation products. The initial value of conjugated diene of sunflower oil was 0.56 which increased to 4.96 after 18 hours of heating. Blending of sunflower oil with antioxidants resulted in a significant decrease in conjugated diene values (Table 6), compared to corresponding control sample. The absorbance of sunflower oil treated with rosemary extract was significantly different from the control oil, and from the oils with the addition of synthetic antioxidant (BHT). At the end of heating periods, the conjugated dienes for the control, BHT, rosemary, fennel, clove and thyme extract oils were 4.96, 2.89, 1.94, 2.26, 2.40 and 2.75; respectively.

Table (6): Effect of aromatic plant oils on conjugated diene value of sunflower oil during heating periods.

Heating periods (hours)	Sunflower oil samples					
	Control	Oil + BHT	Oil + Rosemary	Oil + Fennel	Oil + Clove	Oil + Thyme
Zero	0.56	0.56	0.56	0.56	0.56	0.56
3	0.82	0.72	0.64	0.66	0.69	0.72
6	1.04	0.86	0.76	0.79	0.82	0.85
9	1.68	1.46	0.98	1.15	1.28	1.40
12	2.20	1.82	1.40	1.52	1.70	1.75
15	3.86	1.96	1.78	1.84	1.90	2.22
18	4.96	2.89	1.94	2.26	2.40	2.75

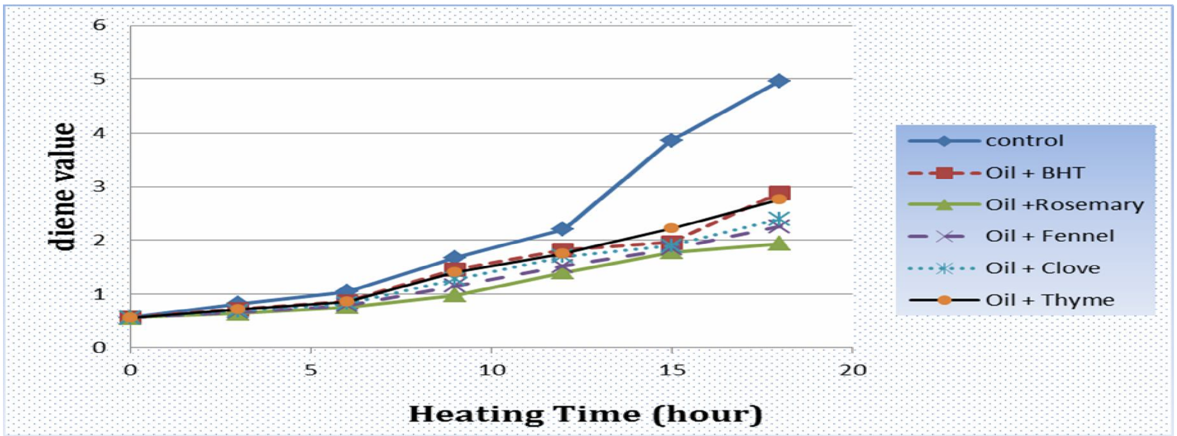

Fig. (4): Effect of aromatic plant oils on conjugated diene value of sunflower oil during heating periods

The same trend of results was noticed for conjugated triene at 268 nm (Table 7 and Fig. 5). The tabulated data showed that there were significant effects on the conjugated triene of sunflower oil with the use of aromatic plant oils, as compared with synthetic antioxidants (BHT). The absorbance for the control oil at the end of heating periods was greater than that for the oil treated with aromatic plant oils extract. For the full treatments of the control, BHT, rosemary, fennel, clove and thyme extract oils, the levels of the conjugated trienes at the end of the heating periods were 3.82, 3.21, 2.4, 2.86, 2.98 and 3.14; respectively. Conjugated dienes and trienes were initially present in the control sunflower oil in small quantities (0.56 and 0.45); respectively. With increases in both with increment heating periods, as has been reported in other studies (Che Man and Jaswir, 2000; Che Man and Tan, 1999 and El-Sayed and Allam, 2003). However the increase in the conjugated dienes was considerably higher compared to the conjugated trienes, which will be specifically due to the high content of linoleic acid in the sunflower oil.

Table (7): Effect of aromatic plant oils on conjugated triene value of sunflower oil during heating periods.

Heating periods (hours)	Sunflower oil samples					
	Control	Oil + BHT	Oil + Rosemary	Oil + Fennel	Oil + Clove	Oil + Thyme
Zero	0.45	0.45	0.45	0.45	0.45	0.45
3	0.62	0.58	0.46	0.52	0.56	0.56
6	0.96	0.80	0.60	0.65	0.72	0.84
9	1.22	1.14	0.86	0.94	0.99	1.24
12	2.36	1.80	1.24	1.49	1.66	1.78
15	2.94	2.63	1.82	2.14	2.46	2.58
18	3.82	3.21	2.40	2.86	2.98	3.14

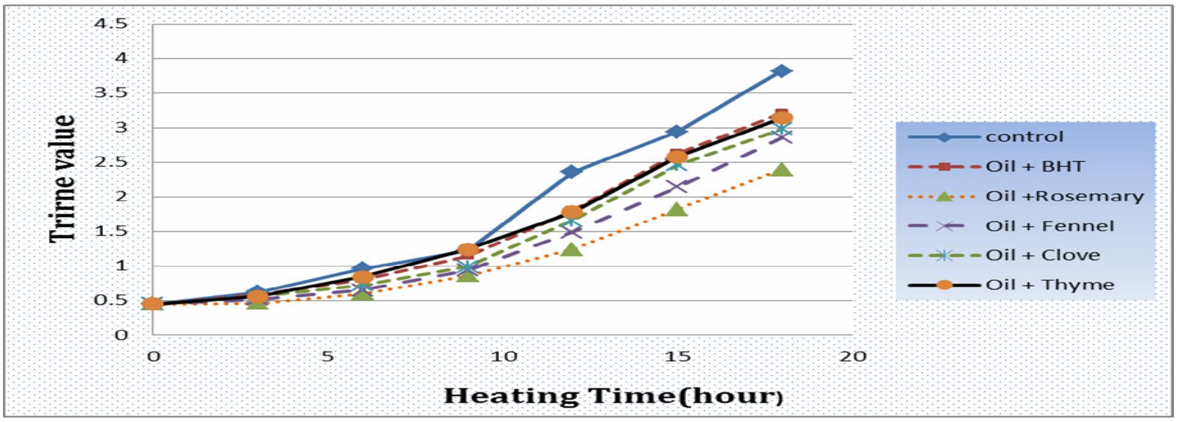

Fig. (5): Effect of aromatic plant oils on conjugated triene value of sunflower oil during heating periods.

From the above mentioned results it could be concluded that the extracted aromatic plant oils possessed antioxidants properties and could be used as alternative synthetic antioxidants with wide food applications.

Moreover, it could be concluded that the studied aromatic plant oils were rich in phenolic components and demonstrated good antioxidant activity.

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تأثير زيوت بعض النباتات العطرية على درجة الثبات الأكسيدي لزيت دوار الشمس أثناء التسخين

عمرو حسين الكاشف ، محمد رشوان عبد العال ، أحمد حامد عبد الغني خليفة ، سومية محمد إبراهيم درويش
قسم علوم وتكنولوجيا الأغذية – كلية الزراعة – جامعة أسيوط

الملخص:

تم الحصول على الزيوت العطرية من أربعة أنواع من النباتات العطرية شائعة الإستخدام وهي على وجه التحديد حصا اللبان والشمر والقرنفل والزعتر . وتم تقدير محتوى هذه النباتات من الفينولات الكلية ، كما تم دراسة النشاط المضاد للأكسدة للزيوت العطرية لهذه النباتات على زيت دوار الشمس مقارنة مع أحد مضادات الأكسدة المخلقة بيوتيليتيد هيدروكسي تولوين وذلك بإستخدام جهاز الرانسيمات كما تم أيضاً تقييم درجة الثبات التأكسدى لزيت دوار الشمس خلال التسخين حتى ١٨ ساعة علي ١٨٠°م ± ٥° م مع استخدام مستخلص زيوت هذه النباتات العطرية مقارنة بمضاد الأكسدة الصناعي.

وأوضحت النتائج أن النباتات العطرية محل الدراسة كانت غنية في محتواها من الفينولات العديدة والتي تراوحت قيمتها من ٩,٣٢ إلي ١٩,٨٢ ملجرام / ١٠٠ جم مقدرة كحامض جاليك على أساس الوزن الجاف. وسجل نبات حصا اللبان المحتوى الأعلى من الفينولات متبوعاً بالشمر ثم القرنفل ، بينما سجل الزعتر المستوي الأقل منها.

وأظهرت النتائج أن فترة الثبات الأكسيدي والمقدرة بجهاز الرانسيمات لزيت دوار الشمس المعامل سواء بمضاد الأكسدة الصناعي أو مستخلصات الزيوت العطرية للنباتات محل الدراسة كانت أطول مقارنة بعينة المقارنة مع وجود فاعلية أكبر لمستخلصات النباتات العطرية. بالإضافة إلى ذلك أدى إضافة الزيوت العطرية إلى زيت دوار الشمس إلى تقليل عملية التأكسد وزيادة الثبات الأكسيدي خلال التسخين لمدة ١٨ ساعة.

بصفة عامة، تعتبر مستخلصات النباتات العطرية موضع الدراسة مصدراً واعداً لمضادات الأكسدة الطبيعية والتي يمكن أن تستخدم في الأغذية لتحل محل مضادات الأكسدة الصناعية لأنها أكثر أماناً وتضيف فوائد صحية للمستهلك.