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ANTIOXIDATIVE EFFECT OF ISOLATED NATURAL ANTIOXIDANTS ON THE STABILITY OF SUNFLOWER OIL DURING HEAT TREATMENTS

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Abstract: The present work was carried out on some new sesame seeds varieties namely: Toshaka 1, Shandaweel 3 and Giza 32 in an attempt to evaluate the utilization of sesame oil as a source of natural antioxidants. Antioxidative effect of isolated natural antioxidants on the oxidative stability of sunflower oil during heating up to 18 hours was evaluated. The acid value of sunflower oil was increased during heating up to 9 hours and then decreased. The oil samples treated with antioxidants had the least amount of free fatty acids, after heating up to 9 hours, which is due to a very low degree of hydrolysis in oil as affected by addition of antioxidants. As heating time was increased, peroxide values increased up to 9 hours, and then decreased. The peroxide values were also less in sunflower oil treated with antioxidants, which was an indication that antioxidants decreased the oxidation of sunflower oil.

The addition of antioxidants to sunflower oil was very effective since the TBA values after 18 hours of heating were significantly less than the values of the oil without adding antioxidants.

Conjugated diene and triene formation in oil samples increased with heating time up to 18 hours. Blending of sunflower oil with antioxidants, resulted in a significant decrease in conjugated diene and triene values, compared with control samples. In general it could be concluded that sunflower oil containing natural antioxidants had a much greater oxidative stability than oils without adding antioxidants. Addition of natural antioxidants could increase shelf life of oils. In addition, natural antioxidants are safe and impart health benefits to the consumer.

Key words: natural antioxidants, sunflower, heat treatments.

Introduction

Sesame oil had achieved a unique status in that it is the most stable naturally occurring liquid vegetable oil. The stability was traced to the presence of natural antioxidants in the crude oil

(Johnson and Peterson, 1974). Sesame oil contains 0.4-1.1% sesamin, 0.3-0.6% sesamolin, and only traces of sesamol, presumably, the superior oxidation stability of sesame oil is due to sesamol, which though present in traces, is capable of generation from sesamolin by hydrogenation, by acid or acid-bleaching earths or other conditions of processing or storage. On the other hand, sesamol might be removed by some bleaching earths or by deodorization (Swern, 1979).

Osawa *et al.* (1985) observed the presence of new types of antioxidants in sesame seeds together with the known antioxidants, sesamol and γ-tocopherol. They reported that the structural elucidation of a new lignan-type antioxidant named sesamolinol, which was more active than Vitamin E, through in vitro ant oxidative assays using rat liver microsome.

Chang *et al.* (2002) investigated the antioxidant activity of ethanol extracts of sesame coat (EESC), they found that the antioxidant activity (91.4%) of 1.0 mg EESC was equal to 1.0 mg tocopherol (90.5%) but was lower than 1.0 mg butylated hydroxyanisole (98.6%) on peroxidation of linoleic acid.

They showed that EESC had an inhibitory effect against the formation of thiobarbituric acid reactive substances. In addition, the chromatographic analysis demonstrated that phenolic compounds and tetranortriter-penoids, which had positive reactions with β-carotene, indicating antioxidant activity, were present in EESC.

Deep fat frying was an important processing procedure used worldwide for the preparation and production of food (Weiss, 1983). 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.

Sesame oil is known to be the most resistant to oxidative rancidity among the vegetable oils, due to the sesame oil containing natural antioxidants such as sesamol and γ-tocopherol (Fukuda *et al*., 1986b). From the practical standpoint, the thermal stability of other vegetable oils would be improved by adding sesame oil.

Kochhar (2000) stated that the natural way of improving oxidative and flavor stability of frying oils and fats is by adding natural ant oxidative components and precursors present in the plant kingdom, such as sesame seed oil. A variety of natural ant oxidative components, present in this oil, comprised sesamolin, sesamol and sesaminol.

Chung and Choe (2001) studied the effect of sesame oil on the oxidation of soybean oil during heating by determining peroxide values, acid values, conjugated dienoic acid contents and fatty acid composition. The obtained results clearly suggested that thermo oxidative stability of soybean oil could be improved through the addition of sesame oil, and higher stability was obtained when sesame oil was blended at a concentration of 30 or 40%.

The present work was carried out in an attempt to evaluate the utilization of sesame oil as a source of natural antioxidants. Anti-oxidative effect of isolated natural antioxidant on the oxidative stability of sunflower oil during heating up to 18 hours was evaluated as well.

Materials and Methods

Materials:Sesame seed samples:

Three varieties of sesame seeds namely: Toshka 1, Shandaweel 3 and Giza 32 were selected in this investigation and obtained from Agricultural Research Center at El Matana during 2004 season.

Commercial oils samples:

Fresh refined, bleached and deodorized sunflower oil without addition of any synthetic antioxidant was obtained from El Nile Company for oils and Detergents, El-Minia, Egypt. The initial characteristics of sunflower oil used in this study was checked by determining acid, peroxide, iodine and TBA values, conjugated diene and triene as described below.

Antioxidants:

Food grade antioxidant of butylated hydroxyl toluene (BHT) was obtained from Sigma Chemical Co., St. Louis, Mo, USA.

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).

Methods:

Thermal treatment of oils:

The two antioxidants used were within the recommended usage levels as previously mentioned. Antioxidants were dissolved in 250 ml sunflower seed oil and kept in brown glass bottles for the following:

a) The sunflower seed 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, iodine and TBA values, conjugated diene and triene to follow the effect of the added antioxidants at high temperature compared to the control.

Physical and chemical properties of oils:

Acid value, iodine value, peroxide value, conjugated diene and triene were estimated according to AOCS (1998). TBA value (thiobarbituric acid number) was estimated spectrophotometery at 532 nm according to Guzman-Chozas *et al*. (1997).

Preparation of crude lignan extract from sesame oil*:*

The extraction of crude lignans from sesame oil were prepared according to the method described by Shyu and Hwang (2002) with some modifications as follows: sesame oil was extracted at ≈ 70°C for 20 min with five-folds of absolute methanol (v/v) in reflective condenser. The extract was then stored at -20°C for 24 h. The upper layer was collected and methanol was removed from this layer. The extract was left overnight yielding more crystals of crude lignans in oily layer. Percentage of crude lignan was calculated as W/V of sesame oil.

Results And Discussion

Antioxidative effect of isolated natural antioxidants on the stability of oils 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 & 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, TBA 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 data tabulated in Table (1) and illustrated in Fig. (1) show the effect of heating time on the acid values of sunflower oil treated with two antioxidants. Acid value reflected the degree of oil hydrolysis and the amount of free fatty acids involved in the heated 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.

The increment in the acid value in control samples was occurred during heating up to 9 hours and then decreased (Fig. 1). The decrement in the acid value after 9 hours might be due to degradation of the formed fatty acids, which could form low molecular weight fatty acids, which are highly

volatile, and/or the formation of the corresponding in alkans via the decarboxylation reaction.

From such data, it could be observed that the oil samples treated with butylated hydroxytoluene (BHT) and crude lignan (extracted from sesame oil) had the least amount of free fatty acids (1.22 and 1.12; respectively), after heating up to 9 hours, which was due to a very low degree of hydrolysis in oils as affected by addition of antioxidants. The difference in activity of the used antioxidants might be accounted on the basis of their chemical structures. These results are in an accordance with those of Aziz & Mohamed (1999), Chung & Choe (2001) and Alsharjabi (2005).

During heat treatment, a progressive decrease in unsaturation was observed in all studied samples by measurement of iodine value (Table 2 and Fig. 2). This decrease 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 & Molero, 2001).

The effect of adding antioxidants (BHT and crude lignan) on iodine value of the sunflower oil was analyzed after heating up to 18 hours. Both antioxidants effectively reduced the oxidation rate in the oil, as detected by increases in iodine values as compared with control samples (Fig. 2) (Sunflower oil without antioxidants). Such results are in good agreement with those reported by Naz *et al*. (2004) and Naz *et al*. (2005).

Table (1): Effect of heating time on the acid values of sunflower oil treated with two antioxidants.

Heating periods (hours)	Sunflower oil samples		
	Control	Sunflower oil + BHT*	Sunflower oil + crude lignan
Zero	0.65	0.63	0.62
3 hrs	1.59	0.86	0.74
6 hrs	1.82	0.94	0.90
9 hrs	2.04	1.22	1.12
12 hrs	1.38	1.64	1.54
15 hrs	0.94	1.96	1.80
18 hrs	0.60	2.12	1.93

* Butylated hydroxy toluene.

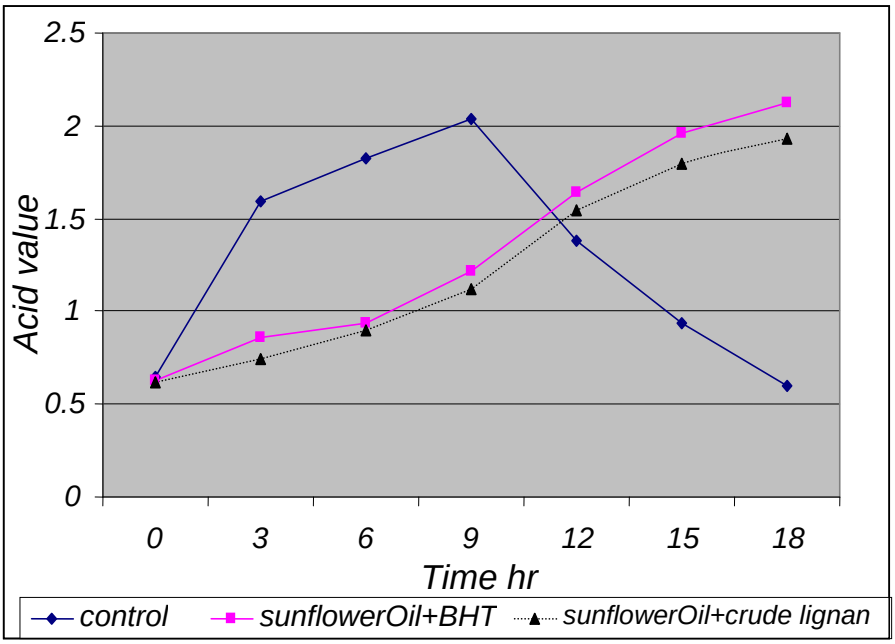

Fig. (1): Effect of heating time on the *Acid value* of Sunflower oil treated with two antioxidants

Table (2): Effect of heating time on the iodine values of sunflower oil treated with two antioxidants.

Heating periods (hours)	Sunflower oil samples		
	Control	Sunflower oil + BHT*	Sunflower oil + crude lignan
Zero	126.82	126.12	126.60
3 hrs	124.61	125.70	125.50
6 hrs	122.32	124.61	124.26
9 hrs	121.40	123.20	122.81
12 hrs	119.80	122.00	120.70
15 hrs	119.20	120.34	120.20
18 hrs	117.30	119.80	118.64

* Butylated hydroxy toluene.

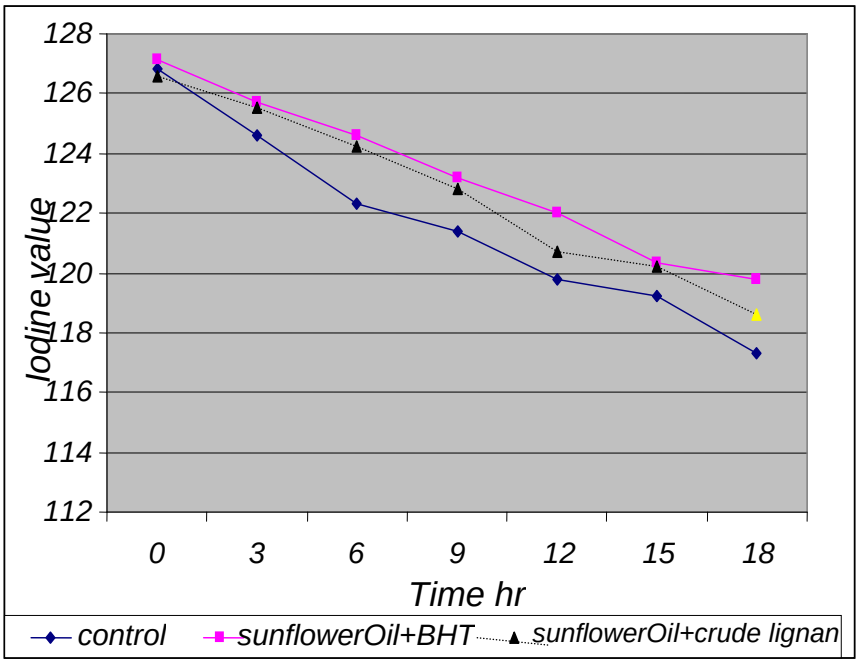

Fig. (2): Effect of heating time on the *Iodine value* of Sunflower oil treated with two antioxidants

Peroxide formation in sunflower oils during heating up to 18 hours is shown in Table (3) and Fig. (3). As heating time increased, peroxide values increased up to 9 hours and then decreased. This was due to the rate difference between peroxide formation and its decomposition. At the beginning of heating, peroxide formation was faster than its decomposition. However, the reverse was the case as the heating proceeded.

The effects of adding antioxidants (BHT and crude lignan) on peroxide value of the sunflower oil were tested after heating up to 18 hours. Both antioxidants effectively reduced the oxidation rate in the oil, as detected by decrease in peroxide values compared to sunflower oil without antioxidant (Fig. 3). Tabulated data showed that continuous exposure of oil to air and light enhanced oxidative changes in the oil and these changes became very fast in heating oil.

Table (3): Effect of heating time on the peroxide values (meq/kg oil) of sunflower oil treated with two antioxidants.

Heating periods (hours)	Sunflower oil samples		
	Control	Sunflower oil + BHT*	Sunflower oil + crude lignan
Zero	3.12	2.86	2.97
3 hrs	5.06	3.92	4.11
6 hrs	8.17	5.81	5.63
9 hrs	10.54	9.20	9.80
12 hrs	6.36	9.66	10.23
15 hrs	5.84	6.70	6.42
18 hrs	4.20	5.10	5.20

* Butylated hydroxy toluene.

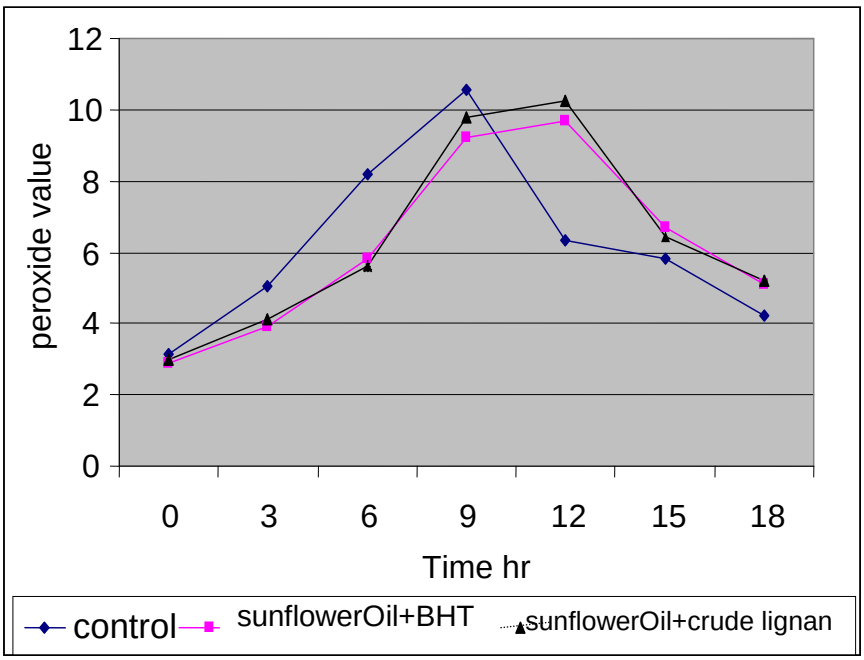

Fig. (3): Effect of heating time on the *peroxide value* of sunflower oil Treated with two antioxidants

The peroxide values were also less in the sunflower oil blended with antioxidants, which was an indication that antioxidants decreased the oxidation of sunflower oil, even if peroxide value determination was not a very good measure of oil oxidation during heating (Stevenson *et al*., 1984).

Table (4) and Fig. (4) revealed that TBA value increased gradually in sunflower oil samples during heating up to 18 hours. This increment in TBA values indicating the formation of carbonyl compounds. Formation of these substances was due to heating in the presence of air. The extent to which these compounds formed might depend on the nature of oil and the heating procedures adopted. These results are in an accord with those of Rehman (1986) and Lee *et al*. (1994). The addition of antioxidants to sunflower oil was very effective since the TBA values after 18 hours of heating were significantly less than the values of the oil without adding antioxidants.

In general, it could be observed that exposure of studied oil to thermal treatment induced pronounced changes in chemical characteristics of this oil. This might be due to the effect of heat on unsaturated fatty acids, which in turn affected the properties of oil.

Fatty acids with conjugated unsaturation absorbed strongly in the region of 230 to 375 nm, (diene unsaturation absorb at 232-234 nm, and triene unsaturation absorb at 268-270 nm). Oil 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 234 nm, which was characteristic of conjugated diene systems (Table 5 and Fig. 5). Conjugated diene formation in oil samples increased with heating time. The initial value of conjugated diene of sunflower oil was 0.62 increased to 4.80 after 18 hours of heating. Blending of sunflower oil with antioxidants resulted in a significant decrease in conjugated diene values (Table 5) compared with control sample. The increase in conjugated dienes was proportional to the sun of hydro peroxides and hydro peroxide decomposition products.

These results are in an accord with those reported in Table (3) and Fig. (3). Such correlations between the conjugated diene values and peroxide values had been previously observed (Noor and Augustin, 1984).

The same trend of results was noticed for conjugated triene at 268-270 nm (Table 6 and Fig. 6).

Table (4): Effect of heating time on TBA values of sunflower oil treated with two antioxidants.

Heating periods (hours)	Sunflower oil samples		
	Control	Sunflower oil + BHT*	Sunflower oil + crude lignan
Zero	0.61	0.58	0.56
3 hrs	0.66	0.60	0.66
6 hrs	0.79	0.71	0.74
9 hrs	0.92	0.89	0.91
12 hrs	1.34	0.92	0.98
15 hrs	1.79	1.45	1.60
18 hrs	1.90	1.82	1.85

* Butylated hydroxy toluene

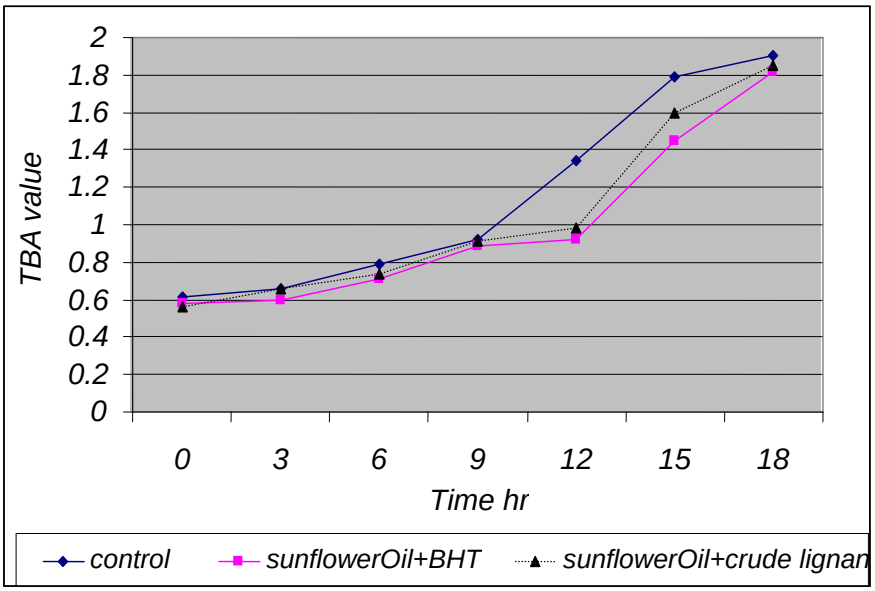

Fig. (4): Effect of heating time on the *TBA value* of Sunflower oil Treated with two antioxidants

Table (5): Effect of heating time on conjugated diene values of sunflower oil treated with two antioxidants.

Heating periods (hours)	Sunflower oil samples		
	Control	Sunflower oil + BHT*	Sunflower oil + crude lignan
Zero	0.62	0.60	0.60
3 hrs	0.74	0.70	0.72
6 hrs	0.96	0.86	0.90
9 hrs	1.34	1.12	1.22
12 hrs	2.20	1.70	1.68
15 hrs	3.55	2.56	2.40
18 hrs	4.80	3.17	3.00

* Butylated hydroxy toluene

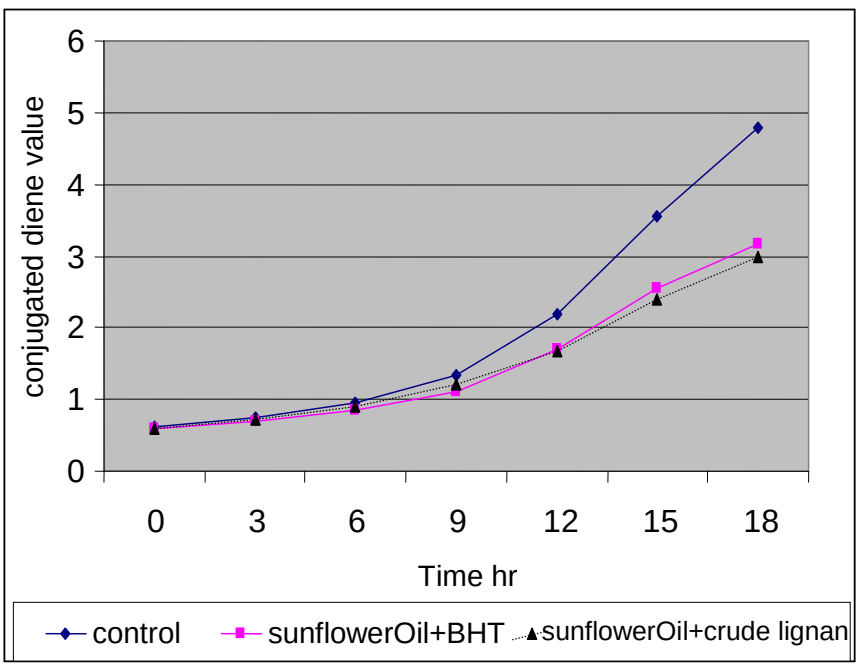

Fig. (5): Effect of heating time on the *conjugated diene value* of Sunflower oil treated with two antioxidants

Table (6): Effect of heating time on conjugated triene values of sunflower oil treated with two antioxidants.

Heating periods (hours)	Sunflower oil samples		
	Control	Sunflower oil + BHT*	Sunflower oil + crude lignan
Zero	0.28	0.26	0.25
3 hrs	0.84	0.78	0.76
6 hrs	1.12	1.04	1.06
9 hrs	1.40	1.20	1.21
12 hrs	2.24	1.82	1.80
15 hrs	2.80	2.03	1.96
18 hrs	3.36	3.12	2.80

* Butylated hydroxy toluene.

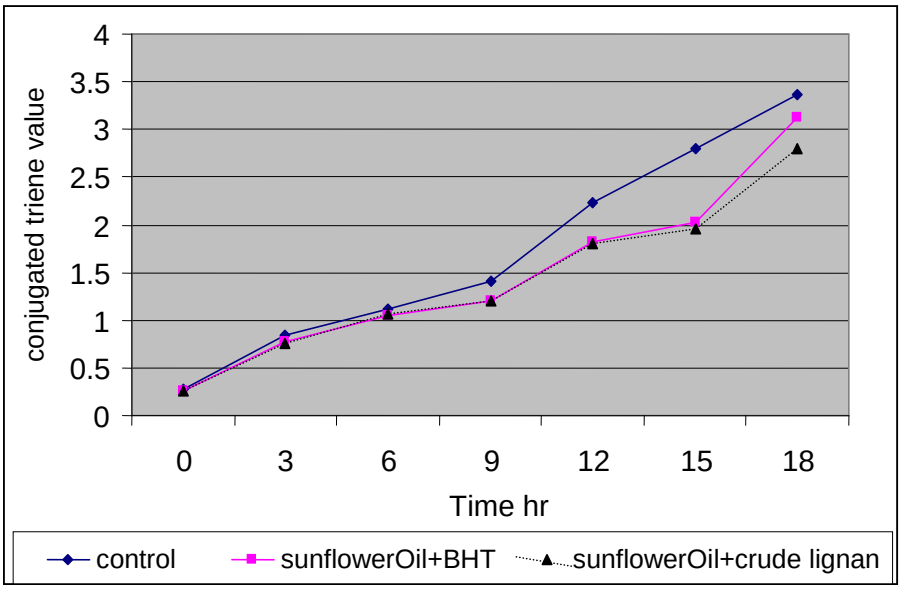

Fig. (6): Effect of heating time on the conjugated triene value of Sunflower oil treated with two antioxidants

From literature, most researchers measured both conjugated diene and triene in different crude or refined edible oils, but they focused only on measurement of alterations during heating, frying or storing of different edible oils on conjugated diene at 232-234 nm, that could be due to the alterations on this UV region was very clear and not overlapping with other cromophore groups of UV spectrum.

On the contrary the other UV regions such 268-270 nm of conjugated triene might be overlapping with other components such as dienals and dienones, which could be produced as secondary breakdown products in oxidation process. Specially conjugated triene formed only on fatty acids containing three or more double bonds, and as mentioned previously the percentage of such fatty acids was very low in studied samples. So we could say that the cromophore groups in this UV region were not well known, also there was much overlapping between more than one cromophore groups.

The above-mentioned results suggested that the unsaponifiables extracted from sesame oil possessed antioxidant properties and could be used as alternative natural antioxidants with wide food applications. No single compound could be considered responsible for this stability. A combination of a number of minor constituents such as tocopherols, seasomol, sesamin, sesamolin and anti-polymerization sterols in sesame oil unsaponifiables could have a synergistic role in increasing the oxidation stability.

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تأثير مضادات الأكسدة الطبيعية المستخلصة من زيت بذور السمسم علي درجة الثبات الأكسيدى لزيت عباد الشمس أثناء التسخين

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أجريت الدراسة على ثلاثة أنواع من بذور السمسم وهى : توشكى 1 ، شندويل 3 ، جيزه 32 . اشتملت الدراسة على تقييم مدى الاستفادة من زيت بذور السمسم كمصدر لمضادات الأكسدة الطبيعية .

وكذلك دراسة تأثير عمليات تسخين زيت عباد الشمس بتقييم تأثير مضادات الأكسدة الطبيعية المستخلصة من زيت بذور السمسم على درجة الثبات الأكسيدى لزيت عباد الشمس أثناء عمليات التسخين لفترات زمنية مختلفة تصل إلى 18 ساعة . ويمكن تلخيص النتائج المتحصل عليها على النحو التالي :

1- حدثت زيادة في قيمة رقم الحموضة لزيت عباد الشمس أثناء التسخين لمدة 9 ساعات ثم تلى ذلك نقص تدريجي في رقم الحموضة حتى نهاية فترة التسخين 18 ساعة . وقد أظهرت نتائج زيت عباد الشمس المعامل بمضادات الأكسدة الطبيعية أن محتواها من الحموضة أقل عن مثيلتها غير المعاملة . وهذا يرجع إلى دور مضاد الأكسدة في تقليل درجة تحلل الزيت أثناء التسخين .

2- حدثت زيادة ملحوظة في رقم البيروكسيد بزيادة فترات التسخين إلى 9 ساعات ، ثم حدث نقص في رقم البيروكسيد باستمرار التسخين . وكان معدل زيادة البيروكسيد في العينات المضاف إليها مضاد أكسدة أقل ، وهذا يعطى دلالة على أهمية مضاد الأكسدة في تقليل حدوث أكسدة للزيت .

3- كان لمضاد الأكسدة دوراً هاماً في تقليل تكوين مركبات الأكسدة الثانوية وبالتالي حدوث انخفاض ملحوظ في قيم الـ TBA مقارنة بالزيوت غير المضاف إليها مضاد أكسدة .

4- أوضحت النتائج زيادة تكوين كل من الـ conjugated diene ، الـ conjugated triene باستمرار التسخين حتى 18 ساعة . في حين كانت الزيادة في الزيوت المعاملة بمضاد الأكسدة أقل. بصفة عامة من نتائج الدراسة السابقة أمكن التوصل إلى أن :

إضافة مضادات الأكسدة الطبيعية إلى الزيوت يؤدى إلى زيادة فترة صلاحية هذه الزيوت بالإضافة إلى أن هذه المضادات الطبيعية آمنة وذات فوائد صحية للمستهلك.و بصفة عامة يمكن القول بأن المركبات الاستيرولية الموجودة في زيت السمسم لها تأثير هام في حماية الزيوت من الأكسدة التى قد تحدث أثناء عمليات التسخين على درجات الحرارة المرتفعة .