

## **EFFECT OF STORAGE PERIODS ON THE STABILITY OF SUNFLOWER OIL TREATED WITH NATURAL ANTIOXIDANTS**

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**Abstract:** This investigation was carried out on some sesame seed varieties, namely: Toshka 1, Shandaweel 3 and Giza 32 in order to study the utilization of sesame oil as a source of natural antioxidants.

The impact of isolated natural antioxidants on the oxidative stability of sunflower oil during storage at ambient temperature for 8 weeks was studied .

The results could be summarized in the following points:

1- Natural antioxidant content (crude lignan) was higher in Shandaweel 3 sesame oil (2.89%) as compared to Giza 32 (2.43%) and Toshka 1 (2.67%) .

2- A gradual increase in acid value occurred during storage of sunflower oils at ambient temperatures. This increment was more pronounced in oils without antioxidants than those containing natural antioxidants.

3- The iodine value decreased gradually in both oils during storage. The rate of decrement in oils without antioxidants was higher than that in oils after adding natural antioxidants.

4- The peroxide value in the stored

samples tended to increase up to a maximal value, then began to decrease . Generally, the rate of peroxide formation in the samples contained natural antioxidants were lower than that of control sample during storage.

5- Increases in TBA values were higher in control sample as compared to sunflower oil that contained natural antioxidants .

6- Conjugated diene and triene contents of sunflower oils increased gradually as the storage time increased.

In general it could be concluded that:

1- Sunflower oil containing natural antioxidants had a much greater oxidative stability than oils without antioxidants. In addition, natural antioxidants are safe and impart health benefits to the consumer .

2- The antioxidants are suitable in their function for increasing oxidative stability at ambient temperature only.

3- The higher efficiency of the natural antioxidants could be due to the stability of these natural antioxidants during storage.

**Key words:** storage periods, stability, natural antioxidants.

## **Introduction**

Oil seeds are commodities of strategic importance as staple food for human nutrition, and the processing of oil seeds to oils, fats and press cakes is also one of the initial steps in the development of agricultural processing. Area under oilseeds exceeds 205 million hectare worldwide, yielding on average 293 million tons per year of oil seeds; current world production amounts to about 105-110 million tons of vegetable oils and 185-190 million tons of oil cakes (ITC, 2001).

The unusual oxidation stability of sesame oil is attributed to the antioxidant effect of some substances, particularly sesamol, which has good potency (Swern, 1979 and Kikugawa *et al.*, 1983). There are some potent antioxidative components other than  $\gamma$ -tocopherol and sesamol in sesame seed oil. These components were identified as sesamol analogue and a sesamin analogue (Fukuda *et al.*, 1985 and Shahidi *et al.*, 1997). Oxidation is a major cause of edible oil degradation, resulting in sensory changes known as oxidative rancidity, frequently responsible for the oil rejection by the consumers (Labuza, 1982). Plastic bottles had been increasingly used for packaging oils, because of their transparency that caused the

product to be more attractive. On the other side, transparency increased light exposure of the oil, thus enhancing oxidation.

The effects of individual plant essential oil on the oxidative stability of sunflower oil were evaluated by measuring the primary oxidation products (peroxide value and conjugated diene hydroperoxides) and generation of secondary oxidation products (anisidine value). Moreover, fatty acid composition of different sunflower oil samples were determined before and after storage period (Abdalla & Roozen, 1999 and Zheng & Wang, 2001). In general, all plant essential oil showed more antioxidant activities in sunflower oil during storage in the dark at ambient temperature than at 60°C.

Fatty acid composition of sunflower oil samples is greatly affected by storage in light, since unsaturated linoleic acid sharply decrease while oleic acid increase, indicating degradation of polyunsaturated fatty acids by the action of light. All edible fats and oils rich in polyunsaturated fatty acids deteriorate under the effect of light exposure (Madsen *et al.*, 1998; Crapiste *et al.*, 1999 and Abo-Ziada, 2002).

Fatty acids with conjugated unsaturation absorb strongly in the region of 230 to 375 nm, (diene unsaturation absorb at

232-234 nm, and triene unsaturation absorb at 268-270 nm). Oils containing linoleate or more highly unsaturated fatty acids are oxidized to conjugated diene systems that can be measured by ultraviolet absorption at 232 nm (Gray, 1978). Oxidation of polyunsaturated fatty acids is accompanied by an increase in the ultraviolet (UV) absorption; lipids with dienes or polyenes show a shift in their double-bond positions due to isomerization and conjugation formation. The resulting conjugated dienes exhibit an intense absorption at 232-234 nm, thus oxidation of polyunsaturated fatty acids is accompanied by increased ultraviolet absorption.

The present work was carried out on seeds of some new sesame seeds varieties namely: Toshka 1, Shandaweel 3 and Giza 32 in an attempt to study the antioxidative effect of isolated natural antioxidants on the oxidative stability of sunflower oil during storage at ambient temperature for 8 weeks.

## **Materials and Methods**

### **Materials:**

#### **Sesame seed samples:**

Three varieties of sesame namely: Toshka 1, Shandaweel 3 and Giza 32 were chosen for this investigation and seeds were obtained from Agricultural Research Center at El Matana

during 2004 season.

#### **Sesame oil samples:**

Sesame oils were extracted by pressing method in Local traditional mill.

#### **Commercial oils samples:**

Fresh refined, bleached and deodorized sunflower oil without addition of any synthetic antioxidant was obtained from El Nile for oils and Detergents Company, 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.

### **Methods:**

#### **Storage of oils:**

Sunflower oil was divided into two portions. The first portion was kept in transparent glass bottles and exposed to diffused light. Where as, the second portion was preserved in similar glass bottles after adding natural extracted antioxidant (crude lignan) with 0.04% ratio. Both sunflower oil samples were placed in screw-capped 350 ml transparent glass bottles, and exposed to diffused light at ambient temperature ( $30\pm 5^{\circ}\text{C}$ ) for 8 weeks. Oxidative stability was evaluated by determining peroxide, iodine and TBA values, conjugated diene and triene were studied as well. The analysis was carried out every week of

storage.

### **Analytical Methods:**

#### **Physical and chemical properties of oils:**

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

#### **Ultraviolet spectroscopy measurements:**

A Shimadzu UV-1601 PC, UV-Visible spectrophotometer, with the UVPC Personal spectroscopy software version 3.91, was used to determine absorptivity at the UV spectrum. The ultraviolet absorbance at 221nm for hydroxynonenal formation as mentioned by Bohnstedt (2005), at 245 nm for malonaldehyde formation as mentioned by Bird and Draper (1984), at 290 nm for acetaldehyde formation as mentioned by Kates (1975), at 232-234 nm for conjugated dienes formation and at 268-270 nm for conjugated triene formation as mentioned by Gray (1978); Chiou (1992) and Vieira & Regitano-Darce (1999), were measured spectrophotometrically. About 0.1 gram of the oil was accurately weighed, dissolved in hexane and transferred quantitatively to a 50 ml glass-stoppered volumetric

flask. The absorbance was corrected to  $E_{1\text{cm}}^{1\%}$  at all wave lengths using the following formula:

$$E_{1\text{cm}}^{1\%} = A/C \times D$$

Where: A is the absorbance of the solution at the specified wave length, C is the concentration of the oil in g/100 ml of the solution, and D is the length of the cell in cm.

#### **Preparation and TLC separation of crude lignans extract from sesame oil:**

The extracts of crude lignans from sesame oil were prepared according to the method described by Shyu and Hwang (2002). Percentage of crude lignan was calculated as W/V of sesame oil. TLC separation of crude lignan extract from sesame oil performed on silica gel 60 plate with solvents: hexane: diethyl ether: acetic acid (70:30:1) according to the method described by Kamal-Eldin *et al.* (1994).

#### **Results and Discussion**

Natural antioxidant component of sesame oil was extracted in this work as crude lignan. The data presented in Table (1) and Figure (1) revealed that crude lignan extract contained eight components with different intensities. The separation was comparable with that previously described by

Kamal-Eldin *et al.* (1994), and the identification of the various components was achieved by comparing of their  $R_f$  values with ordering described by them. Desmethylsterols, sesamin,

sesamol and  $\gamma$ -tocopherol were observed at  $R_f$  0.16, 0.31, 0.39 and 0.47, respectively. Other four unknown components were observed at  $R_f$  0.01, 0.04, 0.59 and 0.89.

**Table (1):** Concentrations of crude lignan extracts from sesame oil

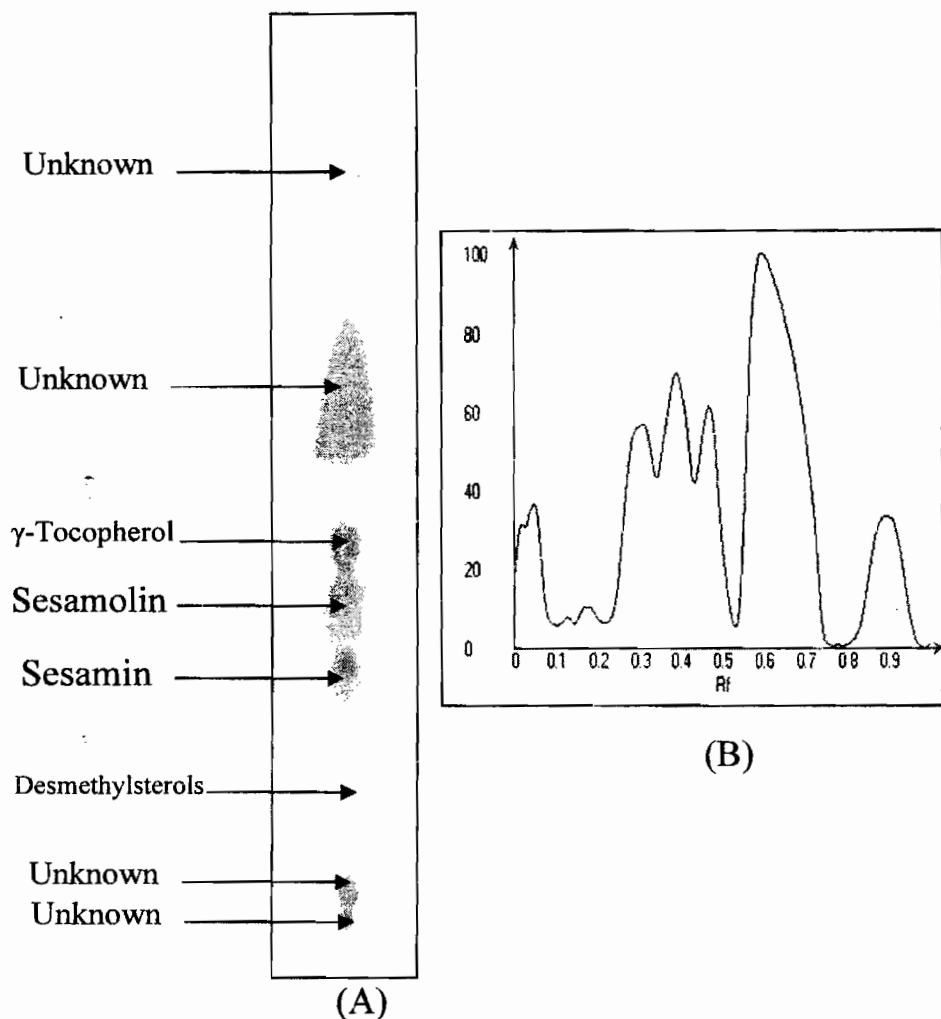
No.	Compound	Peak $R_f$	Concentration%
1	Unknown	0.01	1.77
2	Unknown	0.04	4.26
3	Desmethylsterols	0.16	1.79
4	Sesamin	0.31	14.02
5	Sesamol	0.39	15.00
6	$\gamma$ -Tocopherol	0.47	11.60
7	Unknown	0.59	42.24
8	Unknown	0.89	9.32

The minor of the four known components was desmethylsterols (1.79 %), followed by  $\gamma$ -tocopherol (11.60 %), sesamin (14.02 %) and sesamol (15.00 %), in ascending order. Sesamol could not be detected in this TLC plate, which might be due to its very low concentration in sesame oil. According to Yoshida and Kajimoto (1994) the amounts of sesamin, sesamol, and sesamol were 6824, 5642 and 54 mg/kg in sesame oil, respectively which might explain why sesamol could not be detected by TLC technique. Such results are in general agreement with those reported by Kamal-Eldin *et al.* (1994).

**Effect of storage periods on the stability of sunflower oil treated with natural antioxidants:**

It is well established that deterioration of edible oils could be avoided when the oil is rich with considerable amounts of natural phenolic compounds known as antioxidants. The technological processes which are carried out on these oils to become edible, might have a direct effect on the amount of these natural antioxidants present in oil.

The study was designed to determine the possible decline in the rate of oxidation following addition of crude lignan extracted from sesame seed oil as natural antioxidants. The effects of



**Figure(1):** (A).TLC separation of crude lignan extract from sesame oil on silica gel 60 plate with solvents: hexane: diethyl ether: acetic acid (70:30:1)  
(B). Densitometrical scan of crude lignan extract TLC plate

storage periods at ambient temperatures on the oxidative stability of sunflower oil treated with natural antioxidants are given in Table (2).

Considerable changes were observed in the acid value of sunflower oil during storage at ambient temperatures. As shown in Table (2), the acid value increased from 0.65 and 0.62 to 3.12 and 2.78 after 8 weeks of storage for control oil and (oil + antioxidant); respectively. The increase was considerably higher in oil without antioxidant as compared to oil sample in which natural antioxidants (crude lignan) were incorporated.

The increment in the acid value indicated that light acted as an accelerator of acid formation in the oil. These results are in accordance with those outlined by Adegoke *et al.* (1998) and Byrd (2001).

Tabulated data showed that, the iodine value decreased gradually in both treatments during storage. The rate of decrement in oils without antioxidants was higher than that in oils after the addition of natural antioxidants.

Autoxidation of sunflower oils affected their fatty acids composition, as polyunsaturated fatty acids were oxidized faster than saturated and mono unsaturated fatty acids (Semwal *et al.*, 1996). Oxidation caused a

decrease in the relative percentages of the unsaturated fatty acids and an increase in the relative percentages of the saturated fatty acids. The addition of antioxidants to sunflower oil effectively reduced the oxidation rate in the oil, as detected by relatively low reduction in iodine values (Table 2).

Changes occurring in the peroxide value of sunflower oil during storage are given in Table (2). The peroxide value in the stored samples tended to increase up to a maximal value (10.28) after seven weeks of storage at ambient temperatures, then began to decline in control sample. In general, the natural antioxidant (crude lignan) used in this study showed down the rate of peroxide formation, since peroxide value of samples which contained natural antioxidants were lower (7.90) than that of control sample (8.22) during storage.

The decrement which occurred in the peroxide value after seven weeks of storage could be due to the rate of hydrolysis of peroxidic compounds being higher than the rate of peroxide formation. These results are in agreement with those reported by Raghav *et al.* (1999).

The changes in TBA values of sunflower oil during storage at ambient temperatures are shown

in Table (2). Increases in TBA values were higher in control sample as compared to sunflower oil blended with natural antioxidant (crude lignan). The increase in TBA values was correlated linearly with the storage period, and could be used as an objective parameter for quality deterioration during storage.

Table (2) shows the changes in conjugated diene and triene contents in sunflower oil during storage at ambient temperatures.

Conjugated diene and triene contents of studied oils increased gradually as the storage time increased. Oxidative stability of sunflower oils, based on the changes of conjugated diene and triene contents, were in an agreement with those estimated by peroxide value development.

The data revealed that sunflower oil containing natural antioxidants (crude lignan) had a much greater oxidative stability than oils from sunflower without adding natural antioxidants. These results are in accordance with the results of Yen & Shyu (1989) and Chul Lee *et al.* (2004).

Depending on all the obtained results it could be concluded that the antioxidants are suitable in their function for increasing oxidative stability at ambient temperature only, while they could act as prooxidants or

breakdown into other substances, which could act as prooxidants.

On the other side, the higher efficiency of the natural antioxidants could be due to the stability of these natural antioxidants during storage.

Addition of natural antioxidants could increase shelf-life of oils. In addition, natural antioxidants are safe and impart health benefits to the consumer.

#### **UV Spectroscopy as a tool for observation alterations in stored oils:**

Table (3) shows the effect of storage on hydroxynonenal (at 221 nm), conjugated diene (at 232-234 nm), malonaldehyde (at 245 nm), conjugated triene (at 268-270 nm), and acetaldehyde (at 290 nm), formations in sunflower oils. Tabulated data revealed that all hydroxynonenal, conjugated diene, malonaldehyde, conjugated triene, and acetaldehyde steady increased during storage periods up to 8 weeks at ambient temperature .

Hydroxynonenal (4-hydroxy-2-trans-nonenal) is a major product of the peroxidative decomposition of  $\omega 6$  polyunsaturated fatty acids, such as linoleic acid (C<sub>18:2</sub>) and arachidonic acid (C<sub>20:4</sub>). It absorbs light in the UV region, with a  $\lambda$  max at 221 nm Bohnstedt (2005).



**Table(2):** Effect of storage periods on some characteristics of sunflower oil treated with natural antioxidant.

Storage periods (weeks)	Acid value		Iodine value		Peroxide value		TBA value		Conjugated diene		Conjugated triene	
	Control	Oil + Antioxidants	Control	Oil + Antioxidants	Control	Oil + Antioxidants	Control	Oil + Antioxidants	Control	Oil + Antioxidants	Control	Oil + Antioxidants
0	0.65	0.62	126.82	126.60	3.12	2.97	0.61	0.56	0.62	0.60	0.28	0.25
1	0.82	0.70	125.20	126.40	3.80	3.10	0.64	0.58	0.80	0.68	0.41	0.32
2	1.12	0.92	123.70	126.22	4.64	3.60	0.82	0.65	0.88	0.72	0.68	0.45
3	1.60	1.07	122.60	125.17	5.31	3.96	1.04	0.80	1.25	0.90	0.90	0.68
4	1.96	1.35	120.90	124.31	6.42	4.51	1.42	0.96	1.51	0.98	1.32	0.80
5	2.21	1.80	120.10	123.75	7.96	5.20	1.65	1.20	2.68	1.49	1.67	0.94
6	2.60	2.48	118.80	121.98	9.04	6.10	2.12	1.61	3.40	1.83	2.81	1.37
7	2.80	2.69	117.90	121.32	10.28	6.82	2.67	1.80	4.20	2.79	3.64	1.82
8	3.12	2.78	116.60	120.50	8.22	7.90	3.41	1.92	5.13	3.12	4.15	2.75

**Table(3):** Effect of storage periods on some characteristics of sunflower oil\*.

Characteristics	Sample	0 week	1 week	2 week	3 week	4 week	5 week	6 week	7 week	8 week
Hydroxynonal (at 221 nm)	A**	1.35	1.59	1.84	1.97	2.12	2.18	2.37	2.42	2.72
	B***	1.21	1.30	1.51	1.72	1.86	1.94	2.02	2.12	2.20
Conjugated Diene (at 232-334 nm)	A	2.66	5.38	6.41	7.96	8.12	8.65	8.96	9.20	9.43
	B	2.21	3.55	4.96	5.32	6.40	7.05	7.31	7.64	7.96
Malonaldehyde (at 245 nm)	A	1.30	5.80	8.90	11.41	13.63	16.01	17.56	18.92	20.31
	B	1.22	3.60	4.52	6.31	9.56	10.41	11.62	13.74	15.06
Conjugated Triene (at 268-270 nm)	A	0.82	1.89	2.63	2.96	3.18	4.50	5.16	7.92	8.31
	B	0.60	1.02	1.35	1.90	2.13	3.02	3.64	5.04	6.22
Acetaldehyde (at 290 nm)	A	1.02	1.26	1.37	1.82	2.01	2.22	2.56	3.97	6.80
	B	0.80	0.96	1.10	1.25	1.82	1.96	2.20	2.41	3.42

\* All values calculated as  $E_{1\%}^{1\text{cm}}$ . \*\*A = control oil \*\*\*B = (oil + antioxidant)

The initial value of hydroxynonenal value at 221 nm of the control and (oil + antioxidant) oils was 1.35 and 1.21, increased to 1.97 and 1.72; respectively, after 3 weeks of storage, this value approximately stable or slightly increased during next four weeks of storage, then elevated gradually to reached 2.72 and 2.20 after 8 weeks of storage. The same trend was noticed for conjugated diene at 232-234 nm but it shows sharp increment during first 2 weeks of storage, (from 2.66 and 2.21 to 6.41 and 4.96) for control oil and (oil + antioxidant); respectively, then continued gradually increased to 9.43 and 7.96 at the end of storage after 8 weeks, for oil samples.

According to Bird and Draper (1984), malonaldehyde formation determined by measured absorption at 245 nm. Thus results in Table (3) showed the effect of storage on malonaldehyde formation of studied oils. However with regard to sharp increment and high values of absorption that make us suspect about this values was due to malonaldehyde formation only, specially that malonaldehyde originate from the oxidative decomposition of fatty acids containing three or more double bonds, such as linolenic acid (C<sub>18:3</sub>) or arachidonic acid (C<sub>20:4</sub>).

The values of absorption at 245 nm could be due to the formation of other secondary oxidation products resulted from decomposition of peroxides or hydroperoxides formed in oil oxidation process, therefore this UV region may be helpful for lipid oxidation monitoring.

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 is 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 may 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 not more than 1.6 % in sunflower oils. So we could say that the cromophore groups in this UV region are not well known, also there is much overlapping between more than one cromophore groups.

Table (3) showed the effect of

storage period on acetaldehyde formation of studied oils. Tabulated data revealed that acetaldehyde value increased slightly within first two weeks of storage period, afterward gradually increased up to 6 weeks of storage at , followed by sharp increment due to peroxides and hydroperoxides decompose into secondary oxidation products mainly aldehydes which acetaldehyde is part of this family.

Such findings are in good accordance with Vieira & Regitano-Darce (1998 and 1999).

From these result, it could be concluded that spectroscopical techniques (UV) could be successfully used as a beneficial tools to shed light upon edible oil characteristics and alterations taking place during storage of such oils as well. These techniques could be used instead of the classical methods such as peroxide value and TBA value or other techniques.

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## تأثير فترات التخزين علي درجة ثبات زيت عباد الشمس المعامل بمضادات الأكسدة الطبيعية

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أجريت الدراسة على ثلاثة أنواع من بذور السمسم وهي : توشكى 1 ، شندويل 3 ، جيزه 32 . وأشتملت الدراسة على البذور ، وكذلك الزيوت المستخلصة منها . ولقد تضمنت الدراسة النقاط التالية : تقييم مدى الاستفادة من زيت بذور السمسم كمصدر لمضادات الأكسدة الطبيعية و دراسة تأثير مضادات الأكسدة الطبيعية المستخلصة من زيت بذور السمسم على درجة الثبات الأكسيدى لزيت عباد الشمس أثناء تخزين الزيت فى عبوات شفافة على درجة حرارة الغرفة لفترة ثمانية أسابيع .

ويمكن تلخيص النتائج المتحصل عليها على النحو التالي :

1- كانت نسبة مضادات الأكسدة الطبيعية (crude lignan) المستخلص من بذور شندويل 3 هي الأعلى (2.89%) ، عند مقارنتها بكل من جيزه 32 (2.43%)، توشكى 1 (2.67%) .

2- لوحظت زيادة تدريجية في رقم الحموضة أثناء تخزين زيت عباد الشمس على درجة حرارة الغرفة ، هذه الزيادة كانت أكثر وضوحاً عند تخزين الزيوت بدون اضافة مضاد أكسدة مقارنة بالزيوت المضاف إليها مضاد أكسده .

3- لوحظ تناقص تدريجي في الرقم البيودي أثناء التخزين على درجة حرارة الغرفة . وكان معدل التناقص في الزيوت بدون إضافة مضاد أكسدة أكبر من مثيلتها المضاف إليها مضاد الأكسدة .

4- حدثت زيادة واضحة في رقم البيروكسيد في الزيوت المخزنة تلى ذلك تناقص تلك القيمة بتقدم التخزين . وبصفة عامة فإن معدل تكوين البيروكسيد في الزيوت المحتوية على مضاد أكسدة كان أقل من الزيوت الأخرى غير المحتوية على مضاد أكسدة .

5- كانت الزيادة في قيم الـ TBA أعلى في الزيوت غير المضاف إليها مضاد الأكسدة مقارنة بالزيوت الأخرى المضاف إليها مضاد الأكسدة .

6- حدثت زيادة تدريجية في قيم كل من الـ conjugated diene وكذلك conjugated triene بتقدم عملية التخزين وكانت العينات المحتوية على مضاد الأكسدة أعلى في درجة الثبات بمقارنتها بالعينات الأخرى .

بصفة عامة من نتائج الدراسة السابقة أمكن التوصل إلى :

1- مضادات الأكسدة الطبيعية المستخلصة من زيت السمسم مناسبة جداً لزيادة درجة الثبات الأوكسدي للزيوت عند التخزين على درجة حرارة الغرفة .

2- الكفاءة العالية لمضادات الأكسدة الطبيعية ترجع إلى ثبات هذه المضادات أثناء عملية التخزين .

3- اضافة مضادات الأكسدة الطبيعية إلى الزيوت يؤدي إلى زيادة فترة صلاحية هذه الزيوت بالإضافة إلى أن هذه المضادات الطبيعية آمنة وذات فوائد صحية للمستهلك .