

IMPROVING OF VEGETABLE OILS STABILITY BY USING SOME NATURAL ANTIOXIDANTS PREPARATIONS .

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ABSTRACT

Sunflower oil (Antioxidant free) was treated with black seed and lettuce oil preparations at concentration of 0.1,0.3 and 0.5% , to study the improving stability of sunflower oil toward oxidation and rancidity at ambient , and thermal process at 120 °C for 18 hours. Radical scavenging activity DPPH% results indicated increased the ability of scavenging activity for black seed oil preparation compared to lettuce oil preparation 95.62 and 88.02 % and total phenolic compound contents ranged from 649.79 and 356.73mg/g gallic acid respectively . While results of HPLC indicated ellagic , benzoic and e-vanillic were higher in black seed oil preparation compared with e-vanillic , benzoic and catechol in lettuce oil preparation respectively. Obtained data proved that acid , peroxide and thiobarbituric acid (TBA) values that sunflower oil treated with black seed oil were higher than the other treated with lettuce oil preparation after thermal process. Black seed oil preparation caused the highest oxidative stability (highest induction period) 7 and 6 hours before and after thermal process . The results indicated that the 0.5% black seed oil preparation before and after thermal process and 0.5% and 0.1% from lettuce oil preparation regested the more efficiency before and after thermal process respectively.

Keywords : Vegetable oils , Sunflower oil , black seed and lettuce oil preparations

INTRODUCTION

Vegetable oils contain natural antioxidants and the most common are tocopherols, which are hindered phenolic chain breaking antioxidants. Chain breaking antioxidants are highly reactive with free radicals and form stable compounds that do not contribute to the oxidation chain reaction (El Diwani *et al.*, 2009).

In recent years, there has been an increasing interest in the use of natural antioxidants, such as tocopherols, flavonoids and rosemary extracts for the preservation of food materials . Because these natural antioxidants avoid the toxicity problems which may arise from the use of synthetic antioxidants,such as butylated hydroxy anisole (BHA),butylated hydroxy toluene (BHT) and propyl gallate (PG) (Amarowicz *et al* 2000) and(Bruni *et al* 2004) .

The easiest way is the application of antioxidants directly to foods consumed in the diet. Plants, including herbs and spices, have many phytochemicals which are potential sources of natural antioxidants namely phenolic compounds diterpenes ,flavonoids, tannins and phenolic acids (Dawidowicz *et al.*, 2006).

Natural antioxidants are able to protect from Reactive Oxygen Species (ROS) as well as other free radicals and retard the progress of many chronic diseases and lipid oxidative rancidity in foods. (Gulcin *et al.*, 2003)

There is increasing evidence that consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health

disorders because of the antioxidant activity of these compounds. Since ancient times, herbs and spices have been added to different types of food to improve the flavor and organoleptic properties. Especially popular today is the concept of food that combines nutritional and medicinal benefits, especially antioxidant activity (Goga, *et al.*, 2012.)

Essential oils have been suggested as antioxidants and preservatives in food or even incorporated into foodstuff packaging material for their application as plant and crop protectants. Moreover, promising approaches have been reported using essential oils or components thereof in medicinal products for human or veterinary use. (Tiwari *et al.*, 2009, Kuorwel *et al.*, 2011 and Lang and Buchbauer 2012).

Antioxidant activity of essential oils is another biological property of great interest because they may preserve foods from the toxic effects of oxidants (Maestri *et al.*, 2006). Moreover, essential oils being also able to scavenging free radicals may play an important role in some disease prevention such as brain dysfunction, cancer, heart disease and immune system decline. Increasing evidence has suggested that these diseases may result from cellular damage caused by free radicals (Kamatou and Viljoen 2010).

The term phenolic or polyphenol can be defined chemically as a substance which possesses an aromatic ring bearing one or more hydroxyl substituents, including functional derivatives e.g. ester, methyl ethers, glycosides etc. Most phenolics have two or more hydroxyl groups and are bioactive substances that occur widely in plants. The most active dietary antioxidants belong to the family of phenolic and polyphenolic compounds Shahidi (2000).

So, this work aimed to improve the stability of vegetable oils by using some commercial natural essential oil preparations namely black seed and lettuce oils with different concentrations at 0.1, 0.3 and 0.5% for both fermentation preparation noticing the developing of oil characteristics before and after thermal processes at 120 °C up to 18 hours.

MATERIALS AND METHODS

Materials

Raw materials:

Refined, bleached, and deodorized (RBD) sunflower oil was obtained from Arma Company For Oils at 10th of Ramadan City, Cairo, Egypt. Essential oil preparations namely black seed preparation (*Nigella Sativa*) and lettuce preparation (*Lactuca sativa L.*) were obtained from El-Captain Company for Extracting Natural Oils, Plants and Cosmetics, Al -Oubour City, Cairo, Egypt.

Chemicals:

All chemicals and reagents were purchased from El-Gomhouria Pharmaceutical Company, El-Mansoura City, El-Dakhaleia Governorate, Egypt.

Methods:

Sample preparations:

Preparation of oil samples treated with essential oil preparations:

Sunflower oil samples were treated with (0.1 , 0.3 and 0.5%) from both of black seed preparation and lettuce preparation .All prepared samples were cold stored at $5\pm1^{\circ}\text{C}$ up to further analysis.

Thermal process:

All oil samples including controls samples were heated at 120°C for 6,12 and 18 hours in oven Model WT Binder at Food Industries Dept. Lab. Fac. of Agriculture , Mansoura University. Then oil samples were refrigerated and storage at $5\pm1^{\circ}\text{C}$ till further analysis were carried out.

Analytical method:

Determination of total phenolic compounds:.

The Folin-Ciocalteu method was used to determine of total phenolic compounds (as gallic acid equivalent) using standardized spectrophotometric according to Ivanova *et al.*, (2010) at Food Tech. Res. Institute ,Agricultural Research Center, El-Giza, Egypt.

Determination of Radical Scavenging Activity (DPPH%):.

2,2 diphenyl1-picrylhydrazyl (DPPH %) assay was carried out according to the method described by Brand-Williams *et al.*, (2000) at Food Tech. Res. Institute, Agric. Res. Center, El-Giza, Egypt.

Determination and fractionation of phenolic compounds of essential oil preparations:

Essential oil preparations were determined using HPLC and data were analyzed at Food Tech. Res . Institute, Agric. Res. Center, El-Giza, Egypt using Hewlett packaged software according to the method by (Goupy *et al.*,1999) as follow: 5 g. of sample were mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a $0.2\mu\text{m}$ Millipore membrane filter, then 1-3 ml was collected in vial for injection in to HPLC Agilent 1200 series auto sampling injector ,solvent degasser, ultraviolet (UV) detector set at 280 nm and quaternary HP pump (series 1100).The column temperature was maintained at 35°C . Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1ml/min. Phenolic acid standard from Sigma Co. were dissolved in a mobile phase and injected into HPLC.

Chemical properties of oil samples:

Acid value (AV), free fatty acids (FFA%) and peroxide value (PV) were determined according to the methods described by (A.O.A.C. 2005).

Thiobarbituric acid value(TBA):

Thiobarbituric acid (TBA) value was determined using spectrophotometer, model : SPECTROUV-VISAUTO,UV-2602 and absorbance was measured at 530nm. TBA value was expressed as mg/malonaldehyde/kg oil, according the method described by (A.O.A.C.2005). Using the following equation:

$$\text{TBA} = 7.8 \times \text{O.D.}$$

O.D. = Optical density at 530 nm

Oxidative stability of oil blends:

The oxidative stability of oils blends was determined by Rancimat method according to (A.O.A.C., 2005) at Food Tech. Res. Institute, Agric. Res. Center, El-Giza, Egypt. Induction time refers to the time (h) at the break point of extrapolated of curve by Rancimat apparatus. The stability of oils blends was determined by Rancimat method using Rancimat Metrohm 679 and the induction period (IP) was conducted with Rancimat at 100°C and calculated at 25°C using the temperature coefficient of 2.2 for induction period .

Stabilization factor (F) was calculated from the following equation according to (Marinova and Yanishlieva 1995).

$$F = IP_{inh} / IP_o$$

Where, IP_{inh} =Induction period in the presence of an inhibitor.
 IP_o =Induction period of non-inhibited system..

RESULTS AND DISCUSSION

Total phenolic compounds (TPC) contents (mg/g.) and antioxidants activity (DPPH%) of black seed oil and lettuce oil preparation:

Plant phenolics constitute is one of the major groups of the compounds acting as primary antioxidants or free radicals terminator it was reasonable to determine their total phenolics content (Zeada *et al.*, 2007) and(Safaan,2014) .

Results in Table (1) indicated that the total phenolic compounds showed the percentage of each total phenolic content in essential oil preparation namely black seed oil preparation and lettuce oil preparation .

Data in Table (1) was noted that black seed oil preparation had the highest total phenolic content compared to the other essential oils preparation,it was 649.79 mg/g as gallic acid and the lowest one was dill seed oil preparation being 208.98 mg/g as gallic acid.

Table (1) Total phenolic compounds contents (TPC) as mg/g galic acid and radical scavenging activity (DPPH%) of some essential oils preparations:

	Essential oils preparation				
	Black seed	Ginger	Onion	Lettuce	Dill
TPC	649.79	485.71	304.49	356.73	208.98
DPPH%	95.62	85.17	65.97	88.02	68.25

Data in the same table showed the radical scavenging activity of essential oil preparations was evaluated by DPPH technique which depend on donate hydrogen to free radical and inhibiting the propagation stage in lipids oxidation pathway (Picerno *et al.*,2003 and El-Gammal,2012).

Results in Table (1) showed that highest scavenging activity as DPPH % was detected for black seed oil preparation which reached to 95.62% followed by lettuce oil preparation 88.02% in accordance with the for

mentioned data for black seed oil and lettuce seed oil were selected as natural preparations which use in this investigation.

Fractionation and Identification of studied essential oil preparations.

The amount of phenolic compounds is an important factor when evaluating the quality of essential oil preparations, it involved for their resistance to oxidation and the properties attributed to these antioxidants (Moure *et al.*,2001) .Results in Table (2) indicated that there were a great variation among the components identification of studied essential oil preparations.

Table (2) Fractionation and Identification of chosen essential oil preparations:

Phenolic compounds (mg/g)	Black seed Preparation (BSP)	Lettuce preparation (LP)
Syringic	514.26	843.00
Gallic	ND	119.56
Pyrogallol	166.23	ND
4- Amino-benzoic	3.87	13.01
Protocatchuic	86.09	102.19
Catechein	103.41	175.05
Chlorogenic	448.85	360.77
Catechol	254.01	2144.07
Epicatechein	306.30	822.17
Caffeine	71.39	518.55
P-OH-benzoic	121.68	ND
Caffeic	287.87	257.23
Vanillic	1297.80	416.47
Ferulic	133.02	205.37
Iso-ferulic	761.06	230.60
e-vanillic	2263.27	2504.50
Ellagic	5831.80	899.09
Alpha-coumaric	32.71	20.96
Benzoic	4302.45	2485.31
Salycilic	464.62	ND
3,4,5.Methoxy Cinnamic	ND	ND
Coumarin	986.87	200.01
p-coumaric	2227.76	155.98
Cinnamic	20.51	13.34

ND:None detected

Data in Table (2) showed that black seed oil preparation (BSP) contained 22 compounds of phenolic compounds, the most abundant one being ellagic 5831.80 mg/g concerning to the derivatives with the benzoic and e-vanillic being 4302.45 mg/g and 2263.27 mg/g, respectively while the lowest compounds were p-coumaric, vanillic ,coumarin and iso-ferulic being 2227.76, 1297.80, 986.87 and 761.06 mg/g, respectively.

Regarding with the same table data showed that lettuce preparation (LP) have 20 compounds which could be arranged descending as follows: 4-aminobenzoic, cinnamic, alphacoumaric, protocatchuic, gallic, p-coumaric, catechein, coumarin, ferulic,iso-ferulic and caffeic while the lowest

compounds being 4- aminobenzoic, cinnamic and alphacoumaric being 13.01, 13.34 and 20.96 mg/g , respectively in both of black seed and lettuce oil preparations. While the highest compounds were e-vanillic, benzoic, catechol , ellagic and syringic acid being 2504.50, 2485.31, 2144.07, 899.09 and 843.00 mg/g, respectively these results were in difference with is indential resulted (Safaan, 2014).

The variation of phenolic compounds could be due to the concentration of these compounds which varied between the examined preparations (El-Gammal,2012).

Fat indices chemical of sunflower oil (SFO) treated with studied essential oil preparations during thermal process.

Data presented in Table (3) showed the sunflower oil before addition of essential oil preparations and after thermal procecess had a high level of acid value 0.49 and then highly free fatty acids 0.25 % during thermal process. Both, peroxide and thiobarbitric acid values showed the same elevated intial values 28.84meq/kg oil and 0.63 mg MAD/kg oil but this ratio lowest after addition lettuce preparation when all concentrations proving its effectiveness over black seed preparation same conditions.

On the other hand, all values of AV, FFA% , PV and TBA values were increased during thermal process 120 °C for 18 hours. These results are in accordance with the results of Shahidi and Spurvery (1996), who noticed that autoxidation of oils and the decomposition of hydroperoxides increase in parallel with the temperature increases.

The final thermal process (120 °C for 18 hr), leads to rise the free fatty acid and thiobarbituric acid (TBA) values when concentration 0.1% black seed preparation while 0.3% lettuce preparation was FFA 0.18% and when 0.5 % was TBA value highly 1.38 mg MAD/kg oil because of the high temperature 120 °C used , and the prolonging of thermal process 18hr which helps to increase the hydrolysis process of oil, while the peroxide value was a flactuating factor it was at the focus 0.5% black seed preparation namely 30.82 ml.eqv./kg oil this observations in agreement with those reported by Barbanti et al. (1994) and Kambiz et al. (2003), who stated that the presence of air and water during heat treatment effects the level of oil degradation.

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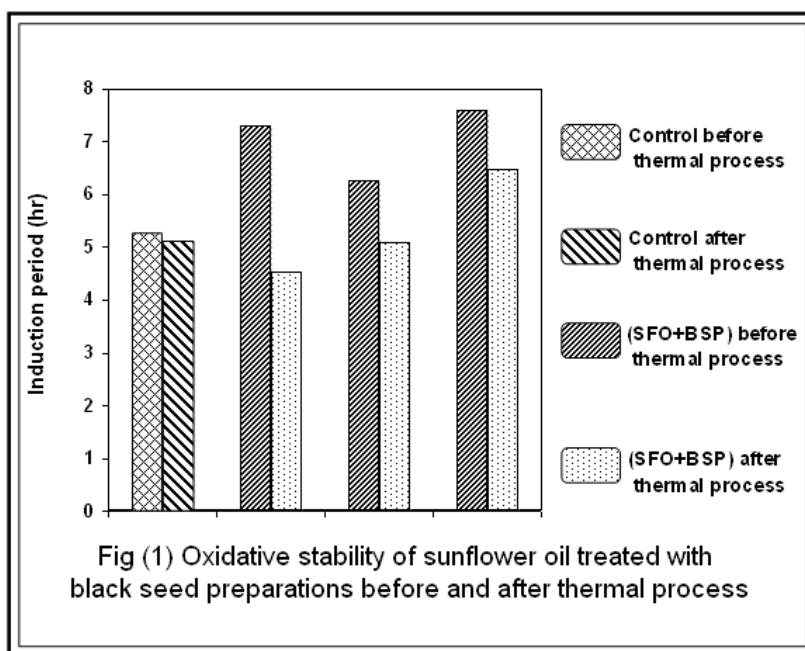
Oxidative stability of sunflower oil treated with essential oil preparation studied:

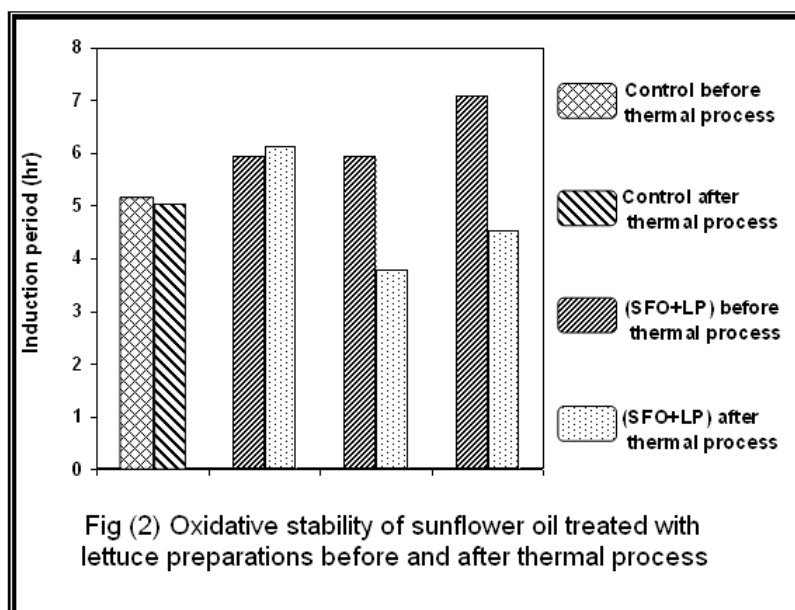
Results from observed in fig (1), showed that all treated oil sample with black seed oil preparation improved the stability against control oil sample. Sunflower oil treated with black seed oil at the concentration of 0.5% have the highest induction period 7.6 hours more than seven months storage. On the after hand, the more concentrations were added, the more oxidative stability were observed.

From obtained results in fig. (2), all these studied concentration of lettuce preparation were preferred than control oil sample partially at concentrations 0.5% with 7.10 hours comperd with 5.17 hours before thermal process.

Sunflower oil with 0.1% lettuce preparation oil showed the highest stability period 6.48 hours six storage months.

Data calculated as the stabilization factor indicated before thermal process Black seed oil preparation were, 1.41, 1.21 and 1.47 and lettuce oil preparation were 1.15, 1.15 and 1.37 respectively also the same direction appear after thermal process that Black seed oil preparation were, 0.90, 1.01 and 1.28 lettuce oil preparation were 1.21, 0.75 and 0.90 respectively. Only 0.1% Black seed preparation showed the negative correlation between preparation concentration and oxidation stabilization of oil. On the other hand 0.3% and 0.5% lettuce oil preparation also showed the negative correlation ship. These were in accordance with (Kiralan *et al.*, 2008).





Changing rate for oxidative stability of sunflower oil treated with studied essential oil preparation during thermal process:

Results from fig. (3) showed change rate for oxidative stability of sunflower.

In fig. (3) Thermal treated led to keep the ability of black seed oil preparations to delay the oxidative rancidity from 21 to 47 % compared to control sample before thermal process .

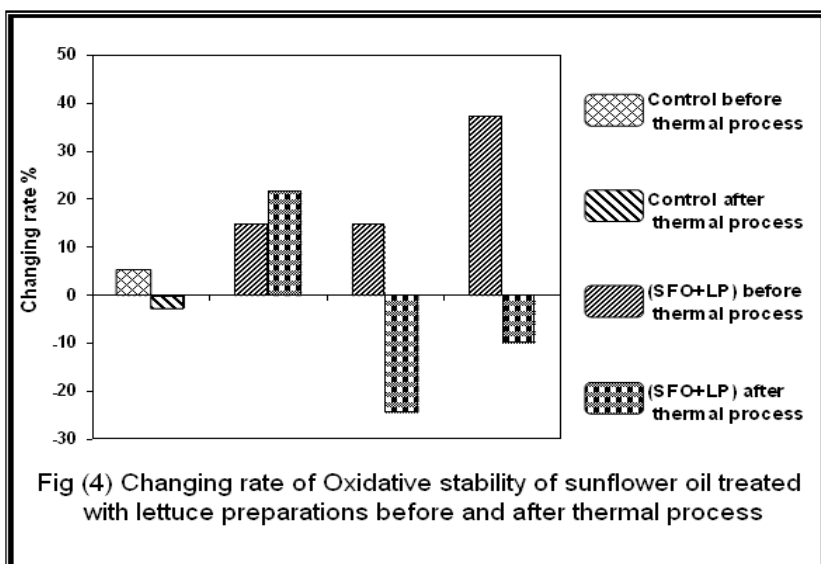
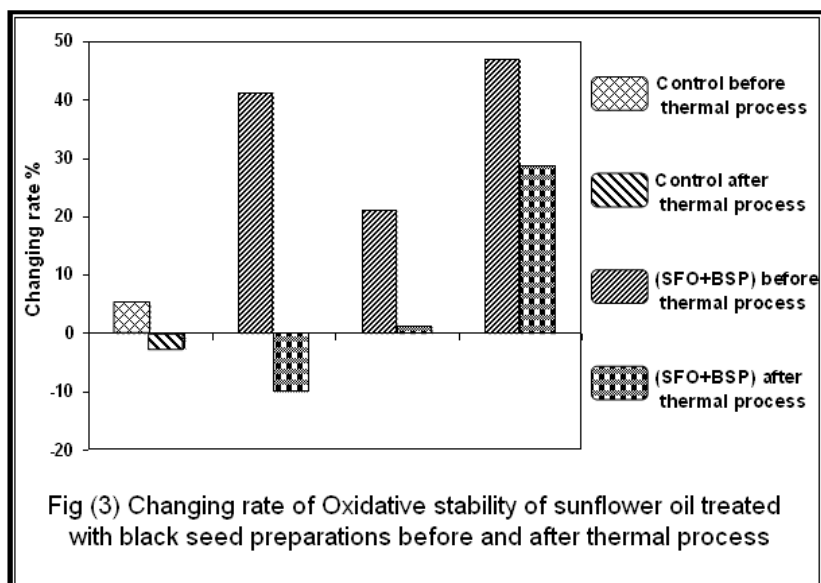
While thermal treatment up to 18 hours at 120 °C led to loss the effectiveness for 0.1% black seed oil preparation to occur the stability. Also, from the same the fig. (3) . At the concentration of 0.5 % the thermal process activate the ability to retard oxidation rancidity around 30% compare to the control sample.

In the case of addition lettuce oil preparations fig . (4). Remained able to improve the oxidative stability around 14 and 37% compared with control sample before thermal process.

At the same fig. (4) only 0.1% lettuce oil preparation showed more improving stability for sunflower oil by 25 % in compare with control sample

While the other sunflower oil with 0.3 % and 0.5 % lost this efficiency after thermal process.

This observation is in agreement with those reported by (Mousavi *et al* ., 2013) who stated that the sunflower containing extract of dill had the highest oxidative induction period or oxidative stability.



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تحسين ثبات الزيوت النباتية باستخدام بعض مستحضرات مضادات الأكسدة الطبيعية .

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تم معالجة زيت عباد الشمس (الخالي من مضادات الاكسده) بكلاً من مستحضر زيت حبة البركة ومستحضر زيت الخس بنسبة (٠.١ , ٠.٣ , ٠.٥ %) وذلك لدراسة امكانية تحسين وزيادة ثبات هذا الزيت ضد عمليات الاكسدة والتزنخ وذلك على درجة حرارة الغرفة والمعاملة الحرارية على درجة ١٢٠ م° لمدة ١٨ ساعة وأظهرت نتائج اختبار (DPPH%) ارتفاع قدرة مستحضرزيت حبة البركة ٩٥.٦٢ % مقارنة (٨٨.٠٢%) لمستحضر زيت الخس.

تراوحت نسبة المواد الفينولية الكلية بين (٦٤٩.٧٩ و ٦٣٥.٧٣) ملغم /غم (حامض جاليك). بينما أعطت نتائج HPLC وجود e-Vanillic , Benzoic , Ellagi بنسبة أعلى في مستحضر زيت حبة البركة مقارنة e-Vanillic , Benzoic , Calechol في مستحضر زيت الخس على التوالي .

وأظهرت النتائج المتحصل عليها لعينات زيت عباد الشمس المعاملة بمستحضر زيت حبة البركة ارتفاع في قيم رقم الحموضة والبيروكسيد وحامض الثيوباربوتريك عن العينات المعاملة بمستحضر زيت الخس بعد المعاملة الحرارية.

أعطت عينات الزيت المعاملة بمستحضر زيت حبة البركة أعلى ثبات تاكسدي على جهاز الرانسيما (٧ و ٦ أشهر) قبل وبعد المعاملة الحرارية.

أوضحت النتائج أن ٠.٥ % من مستحضر زيت حبة البركة قبل وبعد المعاملة الحرارية و ٠.٥ , ٠.١ % من مستحضر زيت الخس أعطت أعلى فاعلية وكفاءة.

Table (3) Fat indices of sunflower oil (SFO) treated with studied essential oil preparations before and after 18 hour thermal process at 120° C

Properties	SFO		SFO + BSP						SFO + LP					
	before	after	0.1%		0.3%		0.5%		0.1%		0.3%		0.5%	
			before	after	before	after	before	after	before	after	before	after	before	after
Acid value (mg KOH/gm oil)	0.07	0.49	0.11	0.46	0.15	0.43	0.15	0.37	0.15	0.29	0.14	0.35	0.22	0.31
Free fatty acid (as oleic acid %)	0.04	0.25	0.06	0.23	0.08	0.21	0.08	0.19	0.07	0.15	0.07	0.18	0.11	0.16
Peroxide value (ml.eqv./kg oil)	4.30	28.84	4.63	41.24	5.33	31.96	4.33	30.82	4.57	47.65	3.83	69.75	10.53	74.30
Thiobarbituric acid (mgmalonaldehyde /Kg oil)	0.50	0.63	0.10	1.04	0.40	0.96	0.30	0.93	0.05	1.16	0.05	1.14	0.15	1.38

(SFO + BSP) Sunflower oil treated with black seed preparation. (SFO + LP) Sunflower oil treated with lettuce preparation.