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THE ABILITY OF OLIVE LEAF EXTRCATS TO ENHANCE THE OXIDATIVE STABILITY OF SUNFLOWER OIL

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ABSTRACT

The present study was undertaken to evaluate the potential of antioxidant activity of olive leaf extracts (OLEs) and compared with other natural or synthetic antioxidants . Different extracts in term of antioxidant properties were studied . Three extracts of olive leaf were obtained by using ethanol , methanol and water .So, the aim of this study was to assess the effectiveness of such extracts and compared it with other antioxidants such as green tea extract , TBHQ , citric acid on the oxidative stability of sunflower oil during storage at an oven at 63±1°C for 0, 5, 10, 15, 20 days. To follow the relative of oxidative deterioration of sunflower oil , as well as deteriming the antioxidative activity of various samples. Oils were analyzed periodically for their peroxide value (PV) , iodine value (IV) , acid value (AV) and thiobarbituric acid (T.B.A.). Analysis of heated sunflower oils demonstrated significant increases in PV and AV and T.B.A . However , IV of the oils were markedly decreased. On the other hand , ethanol extract exhibited the highest antiradical activity among solvents.

Results also showed that the three extracts of olive leaf (i.e. methanolic- , ethanolic- and water- olive leaf extracts) secured protective effect against oxidation of sunflower oil and can serve as substitutes for synthetic antioxidants .

Keywords: Antioxidants, sunflower oil , olive leaf extracts, heat treatment.

INTRODUCTION

The olive is the fruit of an evergreen olive tree that grows in the temperate climate of the Mediterranean region (Soni *et al*., 2006).Olive leaves are a copious by-products deriving from olive tree . The industrial use of olive leaves is limited to animal feed and phytotherpy (Martin Garcia *et al*., 2003). Olive leaves contain high quantities of phenol substances (De Leonardis *et al*., 2008). There is compelling scientific evidence that olive leaf polyphenols are bioactive compounds .

The olive leaf polyphenol composition is similar to that of olive oil. Oleuropein and other secoiridoids are the principle compounds , while simple phenols, enclosed hydroxytyrosol, are present but in lower amounts (Tuck and Hayball, 2002). Olive leaves contain flavonoids such as: rutin flavonol, Luteolin-7-glucoside (Pereira *et al*.,2007) The olive leave extracts (OLEs) was shown to have an antioxidant capacity 400% higher than vitamin C and almost double that of green tea or grape seed extract (Ryan and Robards, 1998).

Lafka *et al*.,(2013) used several solvents,i.e., methanol, ethanol, ethanol:water 1:1, n-propanol, isopropanol and ethyl acetate to extract olive leaf, the most effective solvent was ethanol with optimum phenol extraction conditions 180 min , solvent to sample ratio 5:1 v/w. Ethanol

extract exhibited the highest antiradical activity among solvents and showed the highest antioxidant capacity compared to synthetic and natural food antioxidants such as BHT (Butylated hydroxyanisole), ascorbyl palmitate and vitamin E.

Lipid oxidation is probably the most important factors affecting the shelf life of edible oils. The hydroperoxides produced by lipid oxidation can decompose into various smaller molecules such as aldehydes, ketones, alcohols, and carboxylic acids. Some of these volatile products influence flavor , even at very low concentrations in which both the oil and the food prepared from it become unpalaFigure (Richardsa *et al*., 2005).

Antioxidants have the ability to remove free radicals and reactive oxygen species that damage cellular and tissue structure (Bartosz ,1978). So antioxidants are usually added to fats, oils and food containing fat in order to inhibit the development of off flavours arising from the oxidation of unsaturated fatty acids. The safety of consumer health made the synthetic antioxidants more important, so many studies conducted on antioxidants activity of medicinal plant and edible plants and their application to food preservatives (Tian and White ,1994). Many scientistis fortified natural antioxidants to observe the changes and compare it with the synthetic added antioxidants (Byrd, 2001) .The oxidative stability of oils and fats with added antioxidants can be determined during storage under normal ambient conditions and packing. However, in general, oxidation can take a long time to occur, e.g. a few days to a few months, which is impractical for routine analysis. For this reason, accelerated oxidation or aging tests are conducted. Normally Schaal oven test is used for determination of oxidation of oils (Mahuya *et al*., 2008). Storage of oil samples at high temperatures (oven test) was employed for monitoring oxidative stability of oils and for antioxidant choice.

Sunflower oil is widely used in nutrition as a source of essential linoleic (9-cis,12-cis-octadecadienoic) acid . Sunflower oil more susceptible to oxidation because of higher contents of unsaturated acid i.e., linoleic acid (Steer and Seiler 1990). Oxidation of unsaturated fatty acids is one of the major causes in the development of off-flavor compounds and in the reduction of nutritional value of food products (Hemalatha, 2007). In a study mentioned that fat contained more unsaturation undergo oxidation rapidly (Elliott ,1990). The lipid oxidation of sunflower oil not only can produce rancid odors, unpleasant flavor and discolouration but can also decrease nutritional quality and safety due to degredation products .

Considering that the extractability depends mainly on solvent type and the extraction method , the aim of this study was to assess the effectiveness of methanolic- ,ethanolic- ,and water- olive leave extracts (OLEs) and compare it with other antioxidants such as green tea extract , TBHQ , citric acid on the oxidative stability of sunflower oil during storage at an oven at 63±1^{O}C temperature for 0,5,10,15,20 days.

MATERIALS and METHODS

Preparation of Olive Leaf Extract (OLEs**)**

Olive leaves were collected and put in plastic bags. The plant material was then dried at room temperature and powdered (20 mesh).Ground powdered leaves were extracted in distilled water , ethanol (70% v/v) and methanol (70% v/v) at 20% (w/v) concentration(Lafka *et al*., 2013). The mixtures were mixed individually on rotary shaker for three hours and filtered through whatman and then membrane filter (0.45 um). To obtaine the solid residues of the olive leaf extracts, the extracts were dried in rotary evaporator under lower temperature .

Sample preparation

Olive leaf extracts (300 ppm) , green tea extract , citric acid and synthetic antioxidants TBHQ (at 300 ppm level, for each) were added to virgin sunflower oil before being subjected to oven test to evaluate their capability in retarding the oxidation processes. Control samples bearing no antioxidants were also placed under the same storage conditions.

Oven test

Samples of oil (10 g) were placed in a separate 50 mL open beakers and held in an oven at 63±1°C for up to 0, 5, 10, 15, 20 days. After each storage period, oil samples were immediately analyzed. The temperature of 63°C was used as a rapid method to simulate the storage in real conditions (Besbes *et a*l., 2004)**.**

Chemical Analysis

Determination of peroxide value (PV)

The peroxide value was determined according to A.O.A.C method (2000) A known weight of the oil sample (2.5 g) was dissolved in a mixture consisted of glacial acetic acid: chloroform (30 ml, 3 : 2, v/v) then (1 ml) solution of freshly prepared saturated potassium iodide was added. Distilled water (30 ml) was added then titrated slowly with sodium thiosulphate solution (0.1N).

The antioxidant activity calculated while using the following equation:

100-(PV sample /PV control ×100)

Determination of Acid value

Acid value was determined according the A.O.A.C. method (2000) as follows: A Known weight (2 g) of the oil was dissolved in a neutral ethyl alcohol (30 ml). The mixture was boiled on a water bath for 2 min and then titrated with potassium hydroxide solution (0.1N).

Determination of iodine value

The iodine value (IV) of an oil is a measure of its level of unsaturation. It is defined as the number of grams of iodine that is added to 100 gram of oil (Allen, 1955).

The iodine value was calculated from the following equation,

$1cm^3$ of 0.1N $Na_2S_2O_3$≡ 0.01269 g of iodine

$$IV = 1.26\ (a-b)/w$$

where, a = volume of 0.1N $Na_2S_2O_3$ for the blank, b = volume of 0.1N $Na_2S_2O_3$ for the sample w = weight of the sample., and

Determination of Thiobarbituric acid (T.B.A.)

Thiobarbituric acid value (TBA), the method of Sidwell *et al*. (1954) was conducted to determine the TBA value as follows. A known weight of oil (3g) was dissolved in a carbon tetrachloride (10ml) followed by the addition of TBA reagent (10ml, 0.67% TBA in 50% acetic acid). The mixture was transferred to a separatory funnel and the aqueous layer was drawn into a test tube and immersed in a boiling water bath for 30 min. The absorbance of the developed pink colour was then recorded at 532nm against a blank reagent.

Statistical Analysis

Statistical analysis were performed in triplicate, values are the mean of six determinations ± SD (Kenney and Keeping, 1962) and the ANOVA analysis using the MASTATC program vergion 3 and means were compared using L.S.D.-rang according to (Gomez and Gomez,1984).

RESULTS and DISCUTIONS

Acid value

Acid value is defined by Woollat (1985) as the number of mg of KOH requires to neutralise 1g of fatty acid in an oil. Free fatty acids (FFA) in the heated oil could be considered as a valid oil quality indicator, where the free fatty acids are allowed to go up to >0.5% in the thermal (Gupta, 2005; Rehab and El Anany,2012). Data presented inTable (1) and Fig. (1) show that the acid values of sunflower oil were gradually and significantly (P≤0.05) increased with increasing the period of storage. The acid value of sunflower oil (control) was 8.976 and this value was about 3 times as high as that in sunflower oil treated with Ethanolic-OLE in the end storage. Free fatty acids are formed during oil hydrolysis and thermal process (Wu and Nawar, 1986). On the other hand, methanolic-, and water- olive leave extracts seemed to have similar antioxidant activity as compared with tea extract , TBHQ , and citric acid Table(1) and Fig. (1) .

Table (1): Influence of antioxidants on acid values of sunflower oil during oven test (as meq O_2/Kg)

	Zero	5 days	10 days	15 days	20 days
Control	0.056^{I}±0.01	1.234^{DEFG}±0.2	2.568^{C}±0.1	4.488^{B}±0.4	8.976^{A}±0.5
TBHQ	0.056^{I}±0.01	0.673^{FGHI}±0.1	0.898^{FGHI}±0.3	2.244^{CD}±0.2	4.876^{B}±0.6
Citric acid	0.056^{I}±0.01	0.653^{GHI}±0.3	0.876^{FGHI}±0.4	0.896^{FGHI}±0.2	4.709^{B}±0.3
Green tea ext.	0.056^{I}±0.01	0.401^{GHI}±0.2	0.656^{GHI}±0.2	0.842^{FGHI}±0.3	4.197^{B}±0.2
Water-OLE ext.	0.056^{I}±0.01	0.785^{FGHI}±0.1	0.989^{FGHI}±0.1	2.167^{CDE}±0.4	4.185^{B}±0.1
Methanolic-OLE ext.	0.056^{I}±0.01	0.561^{GHI}±0.1	0.623^{GHI}±0.1	1.708^{CDEF}±0.3	4.086^{B}±0.2
Ethanolic-OLE ext.	0.056^{I}±0.01	0.449^{GHI}±0.3	0.673^{FGHI}±0.2	1.151^{EFGH}±0.2	3.854^{B}±0.1
LSD0.05	1.039				

All Values are means± SD of 3sample .Means in a row with superscripts without a common letter differ, P<0.05.

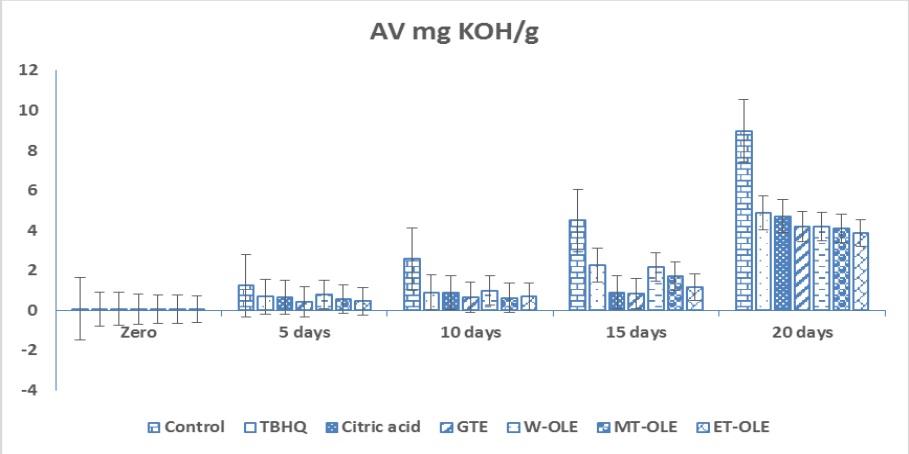


Figure (1): Influence of antioxidants on acid values of sunflower oil during oven test.

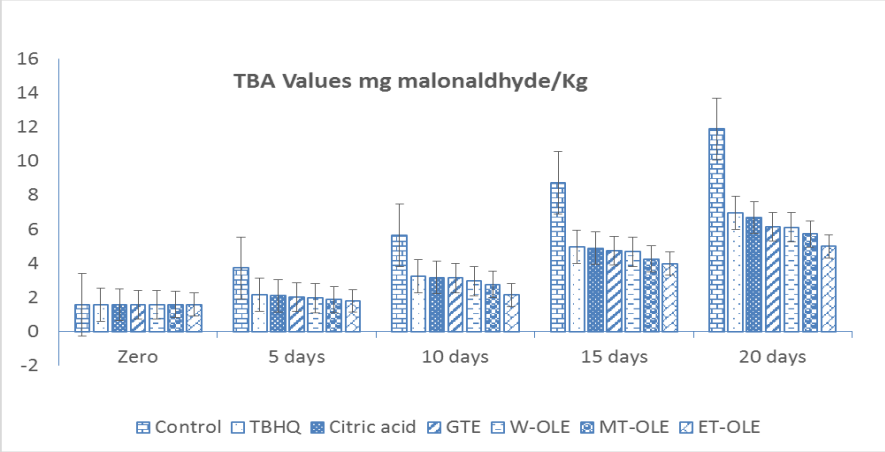
GTE :Green Tea Extract ; W-OLE:Water-OL); MT-OLE:Methanolic-OLE and ;ET-OLE:Ethanolic-OLE.

T.B.A value

Figure (2) show the changes in the TBA values of sunflower oil samples. Results showed that thermal process led to a gradual and significant ($P \leq 0.05$) increases in the T.B.A values and this value was more than 10 times that in sunflower oil at the end of storage period in control sample compared to beginning process .

Results showed also that the rate of production of primary oxidation products was much higher than that of the decomposition of the primary oxidation products and the antioxidants were still effective in protecting sunflower oil against oxidative rancidity. T.B.A values in the control were similar to those in the treatment containing the antioxidants almost as same as the samples with antioxidants in zero time, T.B.A was significantly high in the control (11.868 mg malonaldhyde / kg) compared to the samples with TBHQ (6.945 mg malonaldhyde/kg), citric acid (6.680 mg malonaldhyde / kg), and Green tea extract (6.145 mg malonaldhyde / kg), Methanolic-OLE (5.719 mg malonaldhyde/kg), Ethanolic-OLE (5.008 mg malonaldhyde / kg) and Water-OLE (6.107 mg malonaldhyde / kg) ($P<0.05$) (Figure 3).

This finding clearly indicates the strong effect of the heating period on the formation of secondary oxidation products that were formed during heating process and could be explained by the fact that the primary oxidation compounds (i.e. hydroperoxides) are very unstable and decompose into a series of aldehydes, ketones, hydrocarbons, alcohols, and many more reaction products as the oil oxidation process continues. These carbonyl compounds react with T.B.A reagent to produce T.B.A derivatives that have a high absorbance at 535 nm. (Przybylski and Eskin, 1995).



Figure(2): Influence of antioxidants on TBA values of sunflower oil during oven test (as mg malonaldhyde / kg).

GTE :Green Tea Extract ; W-OLE:Water-OL); MT-OLE:Methanolic-OLE and ;ET-OLE:Ethanolic-OLE.

Peroxide value

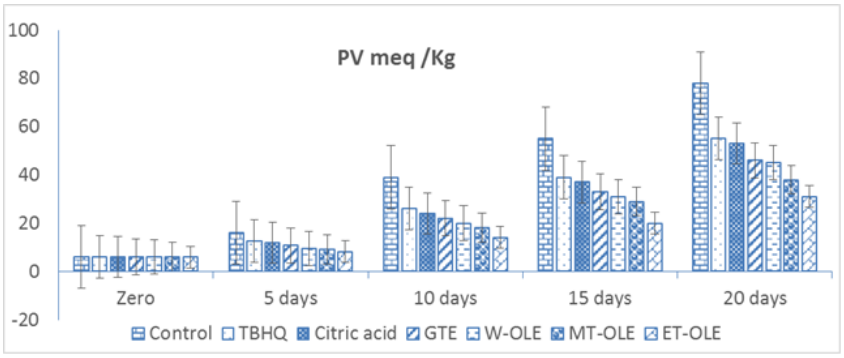
Hydroperoxides are the primary products of lipid oxidation. Therefore, the determination of peroxides can be used as an oxidation index for the primary stages of oil oxidation.

The data presented in Fig.(3) pointed to the changes that took place in peroxidation process of sunflower oil during heat storage. Generally, it was noticed that the PV values were increased gradually in all oil samples during storage; the rate of increase was higher in the control oil sample. This increase in the peroxide value can be attributed to the formation of hydroperoxides, which are the initial products of oxidation. The increase in the PV during storage indicates a decrease of unsaturated fatty acid due to oxidation . However, peroxides are unsFigure compounds particularly under thermal conditions; therefore the peroxides decompose to form carbonyl and aldehydic compounds causing the decrease in peroxide value (Perkins, 1967; Shahidi and Wanasundara, 2002). Thermal process caused forming a molecule of hydroperoxide and releasing peroxy radical (Gupta, 2005).

Addition of the Ethanolic-OLE extract to sunflower oil made the oil more resistant to oxidation , and this gave rise to resistance caused a reduction in the gradient of the increase in the peroxide value (Figure1). In the different treatments of sunflower oil, the oil sample containing Ethanolic-OLE had the lowest peroxide value, while the oil samples which contained Methanolic-OLE and Water-OLE had the second and the third lowest peroxide value, respectively. Rafiee *et al.,* (2011) investigated the antioxidant activities of olive leaf extracts on sunflower oil and compared these effects with those of the synthetic antioxidants .

The peroxide value of the treatments containing Green tea extract and citric acid were higher than that of synthetic antioxidant (TBHQ). Mir-ahmadi *et al.. (2006)* observed that the antioxidant effect of the extract of green tea preventing the oxidation of sunflower oil was greater than that of the synthetic antioxidants .

It is worth to conclude that the phenolic compounds present in OLEs , besides being able to donate hydrogen or electrons , are known as antioxidants due to the intermediation of the Figure radicals which prevent the oxidation of materials, particularly the oxidation of fatty acids and oils that are used in preparing food .



Figure(3): Influence of antioxidants on peroxide values of sunflower oil during oven test (as meq O_2/ KG).
GTE :Green Tea Extract ; W-OLE:Water-OL); MT-OLE:Methanolic-OLE and ;ET-OLE:Ethanolic-OLE.

Antioxidant activity

As shown in Fig. (4) , the antioxidant efficacy of all the antioxidants during storage .The antioxidant activity of Ethanolic-OLE on the five day was 49.27 which reached 60.03 meq O_2 kg -1 of oil on the 20th day, while in the case of other samples , this value decreased from the five day to the 20th day. This can be attributed to the oxidation phenomenon of antioxidants during their storage period. The most potent antioxidant activity was observed for Ethanolic-OLE (Lafka *et al*., 2013)

Iodine value (IV)

The changes in the IV are shown in Figure (5) . IV gives an indication of the number of double bonds in the oil and therefore quantifies the degree of unsaturation in the fatty acids (Oderinde *et al*., 2009). However iodine value can be characterized by a decrease in the total unsaturated contents of the oil and thus is looked upon as an important indicator of deterioration of the oils (Naz *et al*., 2004), it is thus a reflection of the susceptibility of an oil to oxidation.

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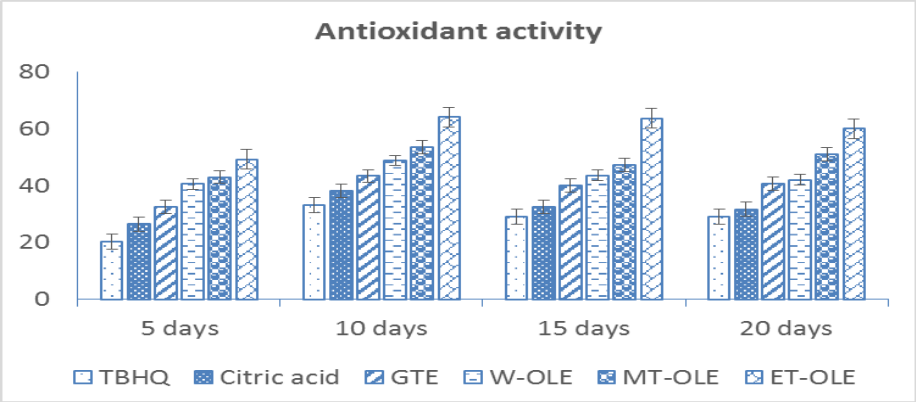
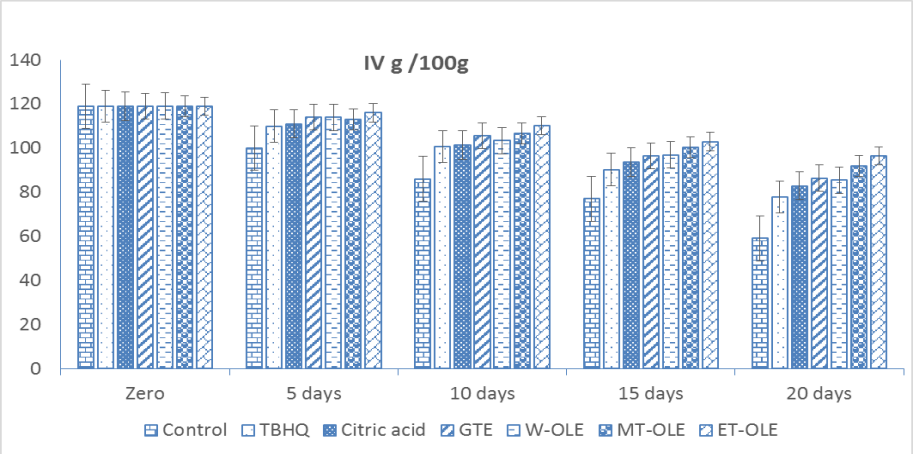


Figure(4): Antioxidant activity for different samples during during oven test. calculated as 100-(PV sample /PV control ×100).
GTE :Green Tea Extract ; W-OLE:Water-OL); MT-OLE:Methanolic-OLE and ;ET-OLE:Ethanolic-OLE.

As shown in Figure (5), the highest significant ($p < 0.05$) change in the IV was shown by sample not treatment, thus indicating that the highest decrease in double bonds occurred due to oxidative rancidity in the during storage. Lesser changes were found in oil samples treated with Ethanolic-OLE (96.5) , Methanolic-OLE (91.8) and Water-OLE (85. 5). IVs of samples treated with Green tea extract were lower than those of samples treated with citric acid and TBHQ.

The obtained results in this study showed obviously the protective role of OLEs as natural antioxidants induced by the presence of sunflower oil resulted in a smaller decrease in the double bonds (Kim and Choe, 2004).



Figure(5) : Influence of antioxidants on iodine values of sunflower oil during oven test.
GTE :Green Tea Extract ; W-OLE:Water-OL); MT-OLE:Methanolic-OLE and ;ET-OLE:Ethanolic-OLE.

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قدرة مستخلصات أوراق الزيتون على تعزيز الثبات التأكسدي فى زيت عباد الشمس

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قسم الكيمياء الزراعية – كلية الزراعة – جامعة المنيا – المنيا – مصر

أجريت هذه الدراسة لتقييم إمكانيات النشاط المضاد للأكسدة من مستخلصات أوراق الزيتون(OLEs)مقارنة مع غيرها من مضادات الأكسدة الطبيعية أو الاصطناعية . تمت دراسة الخصائص المضادة للأكسدة لثلاثة مستخلصات مختلفة من أوارق الزيتون OLEs باستخدام الإيثانول والميثانول و الماء. لذا ، كان الهدف من هذه الدراسة تقييم فعالية هذة المستخلصات و مقارنتها مع غيرها من مضادات الأكسدة مثل مستخلص الشاي الأخضر ، TBHQ ، وحامض الستريك على استقرار الأكسدة فى زيت عباد الشمس أثناء التخزين في فرن عند ٦٣ ± ١ °C ل ٠ و ٥ و١٠و١٥ و٢٠ يوما, لمتابعة التدهور النسبى التأكسدي فى زيت عباد الشمس ، وقد تم تحليل الزيوت بشكل دوري لقيم كل من : بيروكسيد (PV) ، اليود (IV) ، و الحامض (AV) وحمض thiobarbituric (T.B.A) . وبتحليل زيوت عباد الشمس الساخنة أظهرت زيادات كبيرة في PV و AV و T.B.A . وتلاحظ أنخفاض قيمة الرقم اليودى (IV) من الزيوت بشكل ملحوظ . وأيضا أظهرت نتائج مستخلصات أوراق الزيتون الثلاثة تأثيرا وقائيا ضد الأكسدة لزيت عباد الشمس ، ويمكن أن تكون بمثابة بدائل آمنة لمضادات الأكسدة الاصطناعية . وأظهر المستخلص الايثانولي اعلي قدرة في حماية ثبات زيت عباد الشمس مقارنه بالمستخلصات الاخري المستخدمة.

قام بتحكيم البحث

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