*J. Agric. Sci. Mansoura Univ., 27(4),: 2281 - 2290 , 2002*

# PROPOLIS EXTRACT AS A NATURAL ANTIOXIDANT FOR SUNFLOWER OIL

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## ABSTRACT

Propolis extract (PE) at levels of 0.02, 0.05, 0.1, 0.2 and 0.4% (W/W) was evaluated as a natural antioxidant for sunflower oil in relation to some synthetic antioxidant such as Butylated hydroxy toluene (BHT), Tertiary butylated hydroquinone (TBHQ) and citric acid during storage at 50°C/40 days.

Oven stability, peroxide value, TBA and fatty acid analysis were chosen to evaluate the effect of propolis extract as a natural antioxidant for sunflower oil.

Oven stability (OS) of sunflower oil samples treated with PE was improved over the control sample and synthetic antioxidants. It reached to 25 and 27 days at the level of 0.2 and 0.4% (PE), respectively. Addition of PE retarded the development of rancidity of the oil for a period of; at least more than three times of the control sample.

The peroxide value (PV) of sunflower oil samples gradually increased during storage reaching its maximum level after 40 days except the control samples (35 days). TBA values of samples containing PE were lower than those of the control throughout the storage period. The PE at the level of 0.2 and 0.4% were more effective than synthetic antioxidant for retarding oxidative rancidity.

Total saturated fatty acids of sunflower oil samples were slightly increased through 40 days; a trend which corresponding by reduction of total unsaturated fatty acids specially linoleic acid ($C_{18:2}$). A marked decrease in the proportion of ($C_{18:2}$) was observed at the control than all of the other samples. The lowest reduction rate of $C_{18:2}$ (4.8%) was reported for sample containing 0.2% (PE). So, PE at the level of 0.2% or above can be used as a natural antioxidant in prolonging the stability of sunflower oil instead of synthetic ones.

**Keywords** :Natural antoixidant; propolis extract, oven stability, sunflower oil, fatty acid composition.

## INTRODUCTION

It is well known that oxidation of lipids possess real detrimental effects on color, flavor, texture and nutritional value of food. Addition of synthetic antioxidant such as butylated hydroxy anisol (BHA), butylated hydroxy toluene (BHT) and tertiary butylated hydroquinone (TBHQ) can control lipids oxidation. Opinions based on refusing such compounds has been related to health risks; so strict regulations over their use in food products are now upgrading. Subsequently stimulated researches are required for alternative antioxidant sources especially of natural origin such as tocopherols in place of BHA, BHT and TBHQ (Hettiarachchy *et al*, 1996).

Bee propolis which is a sticky amalgamation of plant resins collected by honeybees contain resins, waxes, flavonoids and other unknown materials. It is reported also that propolis have medical, antimicrobial, insecticidal and phytotoxic properties (Johanson *et al*, 1994).

Krol *et al* (1990) and Takeshi *et al* (2001) mentioned that ethanol extract of propolis (EEP) showed remarkable medical properties, including protection of mice against gama irradiation. Its antioxidative effect was attributed to its radical scavenging ability and its antioxidative capacity is partly due to its high content of flavonoids, such as quercetin, flavones, isoflavones, flavonones, anthocyanins, catechin and isocatechin.

Antioxidant activity of propolis towards polyunsaturated lipids systems was tested by (Cengarle *et al.*, 1998), using methyl linoleate as a substrate, and activity was compared with that of alpha-tacopherol. Results indicated that the propolis extracts to realize greater antioxidant effect than alpha- tocopherol. This capability suggests potential use in the preservation of food or high lipid content products.

Sung and Hyaung (1996) studied the effects of propolis on the shelf life of meat products treated with supplement fat during the preservation period. Samples were treated with 0.3% ethanol extract propolis, 0.4% water extract propolis and 0.28% potassium sorbite. Data revealed that propolis extract may acting as a substitute for chemical preservation used in meat products.

The objective aspect of such research was pointed to study the effect of Egyptian bee propolis as a natural antioxidant in preventing or minimizing oxidation of sunflower oil during storage at 50°C in comparison to commercial synthetic antioxidants.

## MATERIALS AND METHODS

### A. Materials :

Honeybee gum which also known as propolis was obtained from El-Kaliobya geographical region. Bleached, deodorizeds sunflower oil was purchased from Egypt Oil and Soap Co., Cairo, Egypt. Butylated hydroxy toluene (BHT), tertiary butylated hydroquinone (TBHQ) and citric acid were of Sigma products.

### B. Methods :

#### 1. Ethanol extract of propolis (PE) :

The "PE" was prepared according to Aza (1989) by slicing about 10 g of propolis sample into fine sections and stirred in 100 ml ethanol (96%) for 4 days in dark-bottle at room temperature (25°C) . The mixture was shacked 15 min for five times daily, then filtered. The obtained extract was evaporated by rotary evaporator at 40°C, followed by weighing the residual sample weighted and transferred quantitatively to 100 ml measuring flask with ethanol (96%). Five concentrations namely 0.02, 0.05, 0.1, 0.2 and 0.4% were prepared from the propolis sample.

#### 2. Performance of storage experiences :

Propolis extract (PE) was added at the level of 0.02, 0.05, 0.1, 0.2 and 0.4% (W/W) to sunflower oil. For comparison, the permitted synthetic antioxidant BHT, TBHQ and citric acid were also added at the 0.02 (W/W)

level. About 50 g of sunflower oil with or without additives were stored in a loosely sealed glass container and placed in standard laboratory oven at 50°C. Oil sample were taken at 5 days intervals for analysis.

**3. Analytical procedures :**

Oven stability (days) was recorded as keeping time, using the procedure of Holley and Hammons (1968), in which each of the samples of sunflower oil (0.5 ml), with or without additives were pipetted in a 30 ml beaker, then placed in convection oven at 50°C until achieving 100 mg increases in weight.

The perceptible rancid odor were followed by a panel of ten judgs to assess off-odor daily in the oil treatment.

Peroxide value (PV) was estimated according to A.O.A.C. (1995). Thiobarbituric acid (TBA) was determined by the method described by Witte *et al* (1970) as a milligrams of malonaldhyed per kilogram of oil sample. Fatty acid methyl ester that prepared according to the A.O.A.C. (1995) were chromatographically analyzed using pye. Unicam PRO.GC for identifying the fatty acids under the following conditions: Column, Poly Ethylene Glycol Adipate 10% (PEGA) (1.5 m. X 4 mm); Detector flame ionization at 300°C, ($H_2$); temperature programming : initial : 70°C, upper : 190°C – Rate 8/min; carrier gas flow, nitrogen (10 ml/min), sample volume, one micro litter.

## RESULTS AND DISCUSSION

Data illustrated in Figure (1) indicated that the oven stability (OS) of the control sample of sunflower oil was 5 days. Meanwhile, addition of different levels of PE increased its "OS" from 5 days up to 10, 14, 23, 25 and 27 days for samples containing the corresponding 0.02, 0.05, 0.1, 0.2 and 0.4% of the PE. Comparing with those reported for samples containing BHT, TBHQ and citric acid, the oven stability was 10, 9 and 8 days, respectively. The "OS" of 0.01 of citric acid as a "synergistics" with 0.01% PE reached up to 14 days. "OS" of sunflower oil sample treated with PE (0.02%) was equal to that of BHT, TBHQ and higher than citric acid. The obtained results agree with those of Yanishieva *et al* (1986) and Takeshi *et al* (2001).

Data in the same table revealed that the development of rancidity for sunflower oil treated by the given concentrations of PE retarded odor changes in all samples as well as BHT, TBHQ and citric acid for a period at least more than 3 times of the control ones. The efficiency increased in terms of number of folds being about four (18 days), five (25 days) and seven (37 day) times than control (5 days) in samples containing 0.02, 0.05, 0.1, 0.2 and 0.4% of PE. Such trend may be due to the higher content of flavonoid glycones and phenolic acid. These observations agree with those obtained by Hemeida and Abd-Al-Fattah (1993) and Menghinello *et al.* (1999).

As shown in Table (1), peroxide values (PV) of sunflower oil samples with or without additives indicated that development of PV occurred at faster rate in control sample than in those treated with the investigated "OS". PV increased progressively during storage and reached its maximum after 40

Table (1): Peroxide values (PV-meq/kg oil) of sunflower oil under different treatments during storage at 50°C/40 day.

| Storage Period (days) | Sunflower oil under different treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | BHT 0.02% | TBHQ 0.02% | Citric acid 0.02% | Propolis extracts (PE) % | | | | | 0.01% PE + 0.01% citric acid |
| | | | | | 0.02 | 0.05 | 0.1 | 0.2 | 0.4 | |
| 0 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 |
| 5 | 7.63 | 3.28 | 5.92 | 5.36 | 4.92 | 4.39 | 4.17 | 2.15 | 2.86 | 4.62 |
| 10 | 9.83 | 7.63 | 12.20 | 9.20 | 9.16 | 9.64 | 8.84 | 7.50 | 3.95 | 9.13 |
| 15 | 24.90 | 17.57 | 20.82 | 20.55 | 27.04 | 26.37 | 20.01 | 12.40 | 9.65 | 25.65 |
| 20 | 25.90 | 18.54 | 32.60 | 30.30 | 27.60 | 26.60 | 23.25 | 16.40 | 15.50 | 26.55 |
| 25 | 32.10 | 32.39 | 32.90 | 44.67 | 31.35 | 44.10 | 33.40 | 23.70 | 21.01 | 41.54 |
| 30 | 50.90 | 40.90 | 44.86 | 50.90 | 39.53 | 49.28 | 38.65 | 27.50 | 21.31 | 49.94 |
| 35 | 84.90 | 68.90 | 67.86 | 63.90 | 52.50 | 56.90 | 56.70 | 42.02 | 25.90 | 63.34 |
| 40 | 80.30 | 68.40 | 69.29 | 68.20 | 67.19 | 68.80 | 65.27 | 60.20 | 48.00 | 65.40 |

days except control sample which presented the maximum PV after 35 days and thereafter decreased as a result of decomposition reactions became prevailing. Addition of PE to sunflower oil sample reduced the formation of peroxide as compared to control due to prolonging the induction period of oil (Poh and Noor, 2000). Regarding the same table, samples were treated by 0.02 and 0.05% PE had a similar PV with those containing BHT, TBHQ and citric acid at the permitted levels in oil. On contrary, at the end of storage, samples containing 0.2 or 0.4% (PE) had a lower PV than all samples 60.20 and 48.00, respectively. So, the use of PE as a natural antioxidant improved the oxidative stability of the oil and had retardatory effect on oil oxidation.

From the aforementioned results it could be concluded that the addition of PE at the level more than 0.02% is more effective in reducing the increment of PV of sunflower oil during storage and prolonging its stability. Yanishlieva *et al* (1986); Cengarle *et al* (1998) and Takeshis *et al* (2002), came to the same conclusion a pattern which confirmed the result.

The TBA value is much more sensitive at earlier stages of oxidation. It is well known that products of oxidation of unsaturated fatty acids, principally linoleic acid, are apparently responsible for the color reaction with TBA. The lipid formed by thermal oxidation included malonaldhyed.

With this view in mind, malonaldhyed as a carbonyl compound formed during oxidation of polyunsaturated fatty acids was evaluated periodically during storage of sunflower oil samples at 50°C treated with different levels of PE as well as BHT, TBHQ and citric acid (Figures 2 and 3). Data elucidate that all PE treatments and synthetic antioxidant had a lower TBA value than the control through the whole storage period. However, the effect on the retardation of TBA construction differed according to the applied PE values. The best result was correlated to samples contained 0.2 and 0.4% (PE) due to their lowest TBA value being 1.05 and 0.54, respectively. Oil sample treated with PE at a concentration 0.02% has the same TBA value which obtained by the synthetic antioxidant at the same concentrations. Addition of 0.01% citric acid as a synergistic to the sample contained 0.01% (PE) retard the increase in TBA value versus storage period reached 1.15 mg malonaldehyde/kg oil at the end of storage. From the same table it could be noticed that PE had a higher effectiveness on the TBA value in treated sample than the control. These results assured that the PE at the level 0.05% is more effective than addition of synthetic antioxidants at the level 0.02%. From the aforementioned data, it was clear that addition of PE at the level up to 0.05% being more effective in retarding oxidation of sunflower oil than that synthetic antioxidants which used at the level 0.02% (Han and Park, 1996; Seung and Young, 1997).

The fatty acids composition of sunflower oil treated by a different level of PE (0.02, 0.1 and 0.2%) in relation to synthetic antioxidant after storage at 50°C/40 days are given in Table (2). The most predominated acids were linoleic acid (62.94%) followed by oleic acid (27.99%); palmitic acid (6.24%) and the ratio between saturated and unsaturated fatty acids was 0.099.

**Table (2): Changes in fatty acids composition of sunflower oil during storage at 50°C/40 days as affected by the investigated treatments.**

| Tested samples | Control | BHT | TBHQ | Concentration of PE | | |
|---|---|---|---|---|---|---|
| | 0 % | 0.02% | 0.02% | 0.02% | 0.1% | 0.2% |
| Identified fatty acids | Zero time | 40 days | 40 days | 40 days | 40 days | 40 days | 40 days |
| **Saturated** | | | | | | | |
| $C_{6:0}$ | 0.102 | 7.128 | 0.023 | 0.073 | 0.034 | 0.335 | 0.070 |
| $C_{8:0}$ | 0.157 | 0.093 | 0.121 | 0.181 | 0.058 | 0.087 | 0.166 |
| $C_{14:0}$ | 1.240 | 1.024 | 0.271 | 0.380 | 0.023 | 0.355 | 0.754 |
| $C_{16:0}$ | 6.241 | 7.920 | 8.680 | 8.860 | 8.791 | 8.670 | 8.009 |
| $C_{18:0}$ | 1.330 | 0.176 | 1.775 | 1.406 | 1.022 | 0.173 | 0.249 |
| Total SFA | 9.070 | 16.341 | 10.870 | 10.900 | 9.928 | 9.620 | 9.248 |
| **Unsaturated** | | | | | | | |
| $C_{18:1}$ | 27.990 | 30.449 | 34.140 | 32.385 | 34.865 | 33.418 | 30.884 |
| $C_{18:2}$ | 62.940 | 53.210 | 54.990 | 56.717 | 55.207 | 58.962 | 59.910 |
| Total USFA | 90.930 | 83.659 | 89.130 | 89.102 | 90.072 | 92.380 | 90.794 |
| **SFA/USFA** | | | | | | | |
| Ratio | 0.099 | 0.195 | 0.121 | 0.122 | 0.110 | 0.104 | 0.101 |

**SFA : Saturated fatty acid.** **USFA : Unsatura**

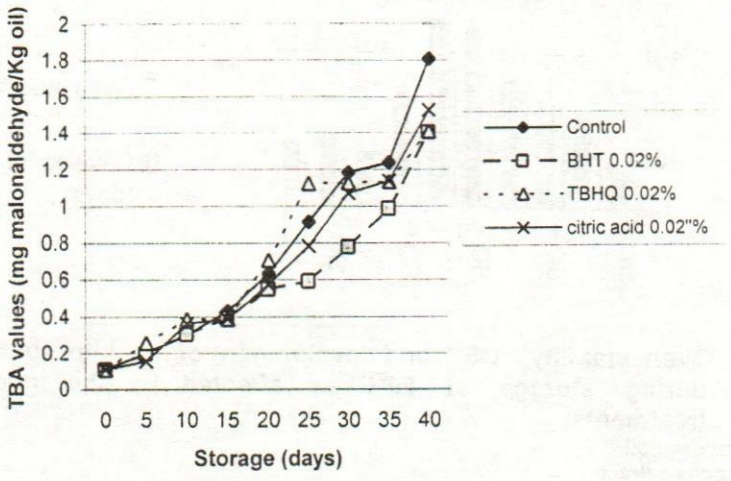


**Fig 1. Changes in TBA values of sunflower oil treated with antioxidants during storage at 50°C for 40 days.**

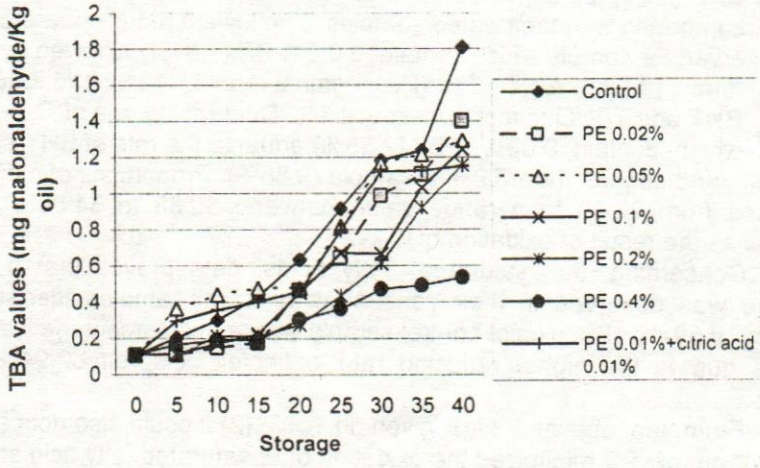


**Fig 2. Changes in TBA values of sunflower oil treated with different concentrations of propolis extract during storage at 50°C for 40 days.**

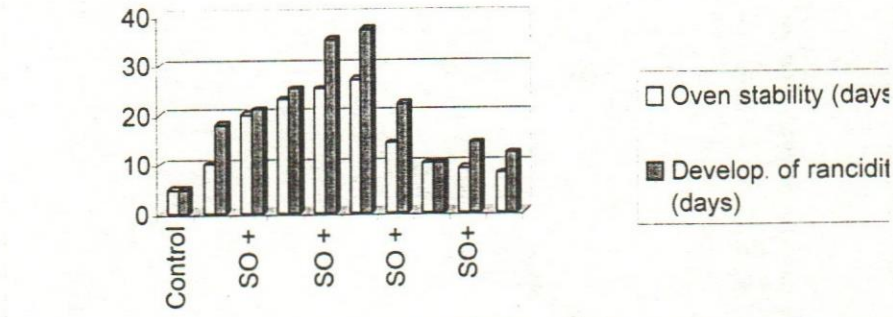


**Fig 3. Oven stability "OS" and development of rancidity sunflower oil during storage at 50°C as affected by the investigated treatments.**

**SO: Sunflower oil.**
**PE: Propolis extract**

After storage at 50°C/40 days the total concentrations of saturated fatty acids increased, which could be related to the decrement in the total unsaturated fatty acids. Addition of PE or synthetic antioxidant to the oil reduced the oxidation of unsaturated fatty acids. A marked decrease in linoleic acid ($C_{18:2}$) of sunflower oil was found to be related to at the control sample comparing with the treated samples. The lowest reducing rate of $C_{18:2}$ was observed for sample which contained 0.2% PE (4.81%) followed by 0.1% (6.32%) then 12.28% for 0.02% (PE) against 15.45, 12.63 and 9.88% for control, BHT and TBHQ samples, respectively. Decreasing rate of $C_{18:2}$ in the sample which contain 0.02% PE (12.28%) equal to the rate of BHT sample (12.63%) and higher than TBHQ sample (9.88%). Proportions of oleic acid increased from 27.99 to a range value between 30.88 to 34.86% for all samples as the result of oxidation of $C_{18:2}$.

Concerning the saturated fatty acids, data proved that a slight increase was observed in their concentration for all samples after storage reaching 9.59-10.99% except control sample where this percentage was only 16.34% due to the higher reducing rate of linoleic acid from 62.94% up to 53.21%.

From the obtained data given in Table (2) it could also noticed that the addition of PE minimized the oxidation of unsaturated fatty acid such as linoleic acid and improved the oil stability for oxidation (Augusten and Berry, 1983; Guillermo *et al*, 1999).

In conclusion propolis as an natural bee product, has an antioxidant activity against oxidation of oils and fatty food. Addition of propolis extract at the level more than 0.2% is more effective in prolonging the stability of sunflower oil than use of BHT, TBHQ and citric acid. Therefore, propolis may be recommended to be used as a natural antioxidant for sunflower oil preservation instead of the synthetic antioxidants.

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## صمغ النحل (البروبوليس) كمضاد أكسدة طبيعى لزيت عباد الشمس

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أجرى هذا البحث لدراسة تأثير استخدام تركيزات مختلفة من المستخلص الكحولى لصمغ النحل (البروبوليس) كمادة طبيعية مضادة للأكسدة على الثبات الأوكسيدى لزيت عباد الشمس أثناء التخزين على درجة حرارة ٥٥°م .

أظهرت النتائج أن استخدام مستخلص البروبوليس أدى الى زيادة زمن الثبات فى الفرن لعينات زيت عباد الشمس بالمقارنة بمضادات الأكسدة الصناعية حيث زاد زمن الثبات خمسة أيام فى حالة عينة المقارنة الى ٢٥ ، ٢٧ يوما فى حالة العينات المحتوية على ٠,٢% ، ٠,٤% المستخلص على التوالى . كما أدى الى تأخير ظهور رائحة التزنخ فى الزيت لمدد تساوى أكثر من ثلاثة الى سبعة أمثال عينة الكنترول ، وقد حدثت زيادة تدريجية فى رقم البيروكسيد لعينات الزيت بزيادة مدة التخزين ووصلت لأقصاها بعد ٤٠ يوما من التخزين ماعدا عينة الكنترول (٣٥ يوم) . وكان أقل معدل زيادة فى رقم البيروكسيد عند استخدام تركيزات من المستخلص مقدارها ٠,٢ ، ٠,٤% .

أشارت النتائج الى أن رقم الثيوباربتيوريك فى عينات الزيت المحتوية على مستخلص البروبوليس كان أقل من عينة الكنترول طوال مدة التخزين وكانت التركيزات ٠,٢ ، ٠,٤% أكثر كفاءة من مضادات الأكسدة الصناعية المستخدمة فى تأخير حدوث التزنخ الأوكسيدى فى الزيت . هذا وقد ثبت من دراسة الأحماض الدهنية لعينات الزيت المعاملة بعد ٤٠ يوما من التخزين حدوث زيادة طفيفة فى تركيز الأحماض الدهنية المشبعة الكلية لعينات الزيت ، وهذه الزيادة كانت مصحوبة بانخفاض فى تركيز الأحماض الدهنية غير المشبعة الكلية وقد ظهر ذلك واضحاً فى حالة حمض اللينولييك حيث كان أعلى معدل انخفاض فى تركيز الحمض (١٥,٤٥%) فى عينة الكنترول مقابل ٤,٨١% فى العينة المحتوية على ٠,٢% من مستخلص البروبوليس .

ومن النتائج المتحصل عليها يمكن القول بأنه يمكن استخدام مستخلص البروبوليس بتركيز ٠,٢% أو أعلى كمضاد أكسدة طبيعى غير تقليدى لإطالة فترة ثبات زيت عباد الشمس بدلا من مضادات الأكسدة الصناعية .