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ANTIOXIDANT PROPERTIES OF OLIVE PHENOLIC COMPOUNDS ON SUNFLOWER OIL STABILITY

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ABSTRACT

The phenolic compounds were extracted from fruits and leaves of Kronakii olive cultivar and fractionated into three major fractions, *i.e.*,free, esterified and residual phenolic acids. These fractions were individually mixed with sunflower oil using different concentrations (100,200 and 400 ppm) to assess their antihydrolytic and antioxidant behaviour. Some fat constants were measured such as acid, peroxide and thiobarbituric acid values for sunflower oil alone and mixed with phenolic components during storage at room temperature. The antihydrolytic and antioxidant phenomena of olive phenolic compounds were compared with BHT activity as a common synthetic antioxidant.

The results demonstrated that total and free polyphenols obtained from both leaves and fruits of Kronakii olive cultivar possessed an antihydrolytic and antioxidant activities and these phenomena were increased by increasing their concentrations. At 400 ppm level, the aforementioned phenolic compounds exhibited remarkable antihydrolytic and antioxidant activities and were superior to that of BHT in retarding sunflower oil stability.

Key words: *olive fruits and leaves , polyphenols, quality assurance tests , rancidity , sunflower oil.*

1.INTRODUCTION

Lipid peroxidation causes various damages not only in living organisms but also in foods. In order to retard undesirable changes in lipids due to oxidation it is necessary to add antioxidants to food products before use (Farag *et al.*, 1990 and Buck and Edwards 1997). The most common antioxidants are tocopherols and synthetic phenolic compounds such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT). The use of BHT or BHA in food has been decreased because of their suspected action as promoters of carcinogenesis, as well as the general consumer rejection of synthetic food additives (Namiki, 1990). In addition, BHA and BHT are characterized by high volatility and instability at elevated temperatures (Dapkevicius *et al.*, 1998). Therefore, there is a great interest in substituting the aforementioned synthetic antioxidants by other natural antioxidants (Farag *et al.*, 1990, Pratt, 1992 and Tsimidou and Boskou, 1994).

Among the most important natural antioxidants are tocopherols and ascorbic acid. Tocopherols are potent inhibitors to lipid peroxidation *in vivo* but they are less effective than BHA or BHT as food antioxidants (Tsimidou and Boskou, 1994). Melted beeswax and its unsaponifiable constituents were mixed with butter oil or refined cottonseed oil to study their hydrolytic and oxidative effects (Farag *et al.*, 1993). The unsaponifiables at different levels exhibited an anti-hydrolytic and antioxidant effects on butter oil and refined cottonseed oil, respectively. Xinchu *et al.* (1998) found that the petroleum ether, acetic acid, ether and alcohol (95%) extracts of different parts of *Salvia plebeia* induced an antioxidant activity. Thyme and cumin essential oils were used to prevent cottonseed oil and butter rancidity during storage at room temperature (Farag *et al.*, 1990). In addition, thyme and clove essential oils are quite safe and can be applied practically as natural antioxidants for lipids (Farag *et al.*, 1991). Charai *et al.*, (1999) studied the effect of essential oils obtained from certain aromatic plants as natural antioxidants for olive oil. Their results showed a wide variation in the antioxidant activity of the essential oils and the highest activity was observed with *Thymus broussonetti* essential oil.

The aim of the present work was to extract the total polyphenols from leaves and fruits of Kronakii olive fruits and leaves and fractionate them into three fractions, *i.e.*, free, esterified and residual phenolic compounds. These fractions were individually added to sunflower oil in an attempt to increase its stability and to compare their antioxidant activity with BHT.

2.MATERIALS AND METHODS

2.1.Source of olive leaves and fruits

The ripe oilve leaves and fruits of Kronakii cultivar were collected during the season 2000 from the Horticulture Research Institute, Ministry of Agriculture, Giza, Cairo, Egypt.

2.2.Solvents and Reagents

All solvents were distilled before use. Butylated hydroxy toluene (BHT), and thiobarbituric acid (TBA) were purchased from *Sigma Chemical Co.,* St Louis, MO, USA; and *Gerbsaure Chemical* Co. Ltd., Germany, respectively.

2.3.Sunflower oil

Refined sunflower oil was obtained from Cairo Oil and Soap Co., El-Ayat, Giza, Egypt. The oil peroxide and acid values were 2.02 meq/kg and 0.36 mg KOH/1g oil, respectively.

2.4.Extraction of olive polyphenols

Olive fruit and leaf polyphenols were extracted with ethanol followed by centrifugation at 1500 xg for 15 min. The ethanolic extracts were dried over anhydrous sodium sulfate and evaporated to dryness (Kanner *et al*., 1994).

2.5.Fractionation of polyphenols

Free, esterified and residual phenolic fractions were separated from olive fruits and leaves of Kronakii cultivar according to the method of Dabrowski and Sosulski, (1984). Free phenolic acids were initially extracted with tetrahydrofuran containing NaBH4 (0.5%) followed by extraction of soluble phenolic esters with a mixture of methanol : acetone : water (7:7:6 , v/v/v) . Alkaline hydrolysis was

employed followed by extraction with a mixture of diethyl ether: ethyl acetate: tetrahydrofuran (1:1:1, v/v/v) to obtain the insoluble bound phenolic acids.

2.6.Oxidation systems

Different concentrations of total, free, esterified and residual phenolic compounds (100,200, 400 ppm) and BHT (200 ppm) were individually added to sunflower oil. The antihydrolytic and antioxidant activities of each phenolic fraction were followed up by the determination of the acid, peroxide and thiobarbituric acid tests daily over a period of 20 days. These values were used to compare the effectiveness of the phenolic fractions on sunflower oil stability.

2.7.Quality assurance methods

The chemical measurements of the following were determined using Standard American Oil Chemists Society methods (A. O. C. S.-1985) indicated in the parentheses, acid value (cd 3 a. 63), and peroxide value (cd 8.53). The secondary oxidation products were determined by the thiobarbituric acid (TBA) test (Ottolenghi, 1959). Three replications were run for each parameter during sunflower oil storage and the mean values are presented in the text.

2.8.Statistical analysis

The data of quality assurance tests were subjected to analysis of variance with a randomised complete block design to partition the effects of different parameters (Steel and Torrie, 1980). The simple regression coefficient (reaction slope) for acid value was statistically calculated.

3.RESULTS AND DISCUSSION

There is currently a great worldwide interest in finding new and safe antioxidants from natural sources to prevent food rancidity. The present study was focused on olive polyphenols which do not induce undesirable odour or taste and are separated from olive leaves and fruits of Kronakii olive (very cheap natural source) into 3 major fractions, *i.e.*, free, esterified and residual phenolic compounds. These fractions beside the total polyphenols were added individually to

sunflower oil at various concentrations in order to extend the shelf-life of the oil.

The antioxidant and antihydrolytic activities of olive various phenolic components were assessed by assesing the changes in some fat constants (acid, peroxide and thiobarbituric acid values). An experiment was performed where sunflower oil was catalyzed by BHT (200 ppm) in order to compare the antioxidant efficiency of the olive phenolic compounds under study with the most commonly used synthetic antioxidant material. It has been reported that synthetic antioxidants (BHT, BHA and PG) are added at concentrations of 100-400 ppm to fats and oils to suppress the development of peroxides during food storage (Allen and Hamilton, 1983). Therefore, the phenolic fractions were added at concentrations of 100, 200 and 400 ppm to sunflower oil.

Figure (1) shows the changes in acid values of sunflower oil mixed with phenolic fractions obtained from leaves and fruits of kronakii olive cultivar and BHT during storage at room temperature. The acid values for the systems consisted of sunflower oil alone and sunflower oil catalysed by BHT, total, free, esterified and residual phenolic fractions were linearly increased over the storage period. In other words, there was a linear relationship between the acid values and storage period. In order to evaluate the effectiveness of the added phenolic materials to sunflower oil, the reaction slope of the acid value curves was used as a guide in this context. Accordingly, the slope values for the acidity of sunflower oil alone and catalysed by BHT (200 ppm), total (100,200,400 ppm), free (100, 200, 400 ppm) phenolic fractions of Kronakii leaves were; 0.5, 0.3, 0.4, 0.3, 0.1, and 0.4, 0.3, 0.2, respectively. Also, the slope values of the acid value curves representing the esterified (100, 200, 400 ppm) and residual (100, 200, 400 ppm) phenolic fractions of Kronakii leaves were the same (0.5). A slope value greater than one-half indicates pro-hydrolytic effect whereas a value lower than 0.5 demonstrates an anti-hydrolytic activity. Hence, the systems containing BHT, total and free phenolic fractions exhibited an anti-hydrolytic activity. Conversely, esterified and residual phenolic fractions at various levels caused non-significant anti-hydrolytic activity on sunflower oil.

The slope values of the sunflower oil acid values using the phenolic fractions from Kronakii fruits were nearly similar to that

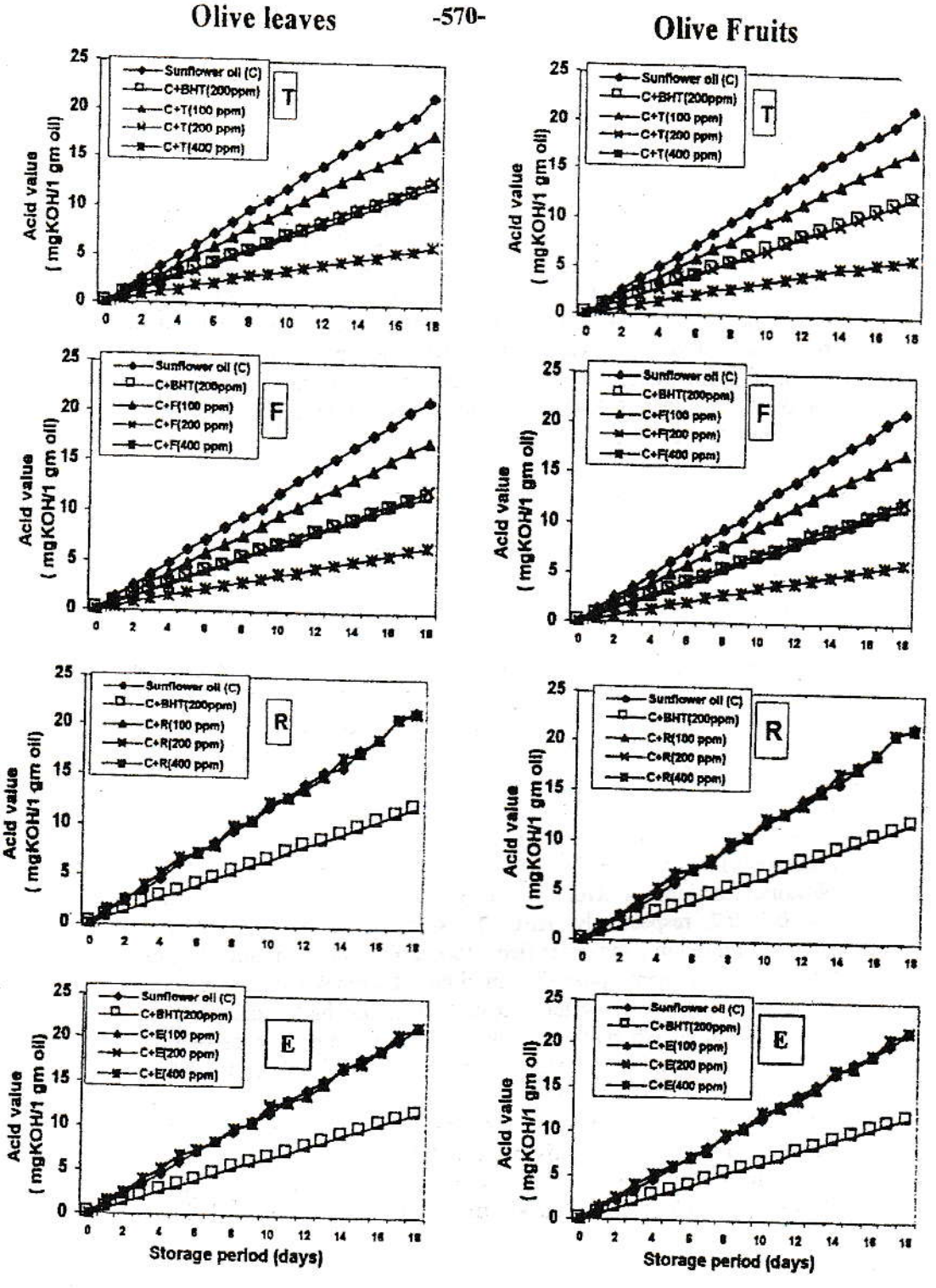

Fig.(1):Effect of total (T), free (F), residual (R) and esterified (E) polyphenolic compounds of Kronakii olive fruits and leaves on the acid value of sunflower oil.

obtained from the Kronakii leaves. In general, total and free polyphenols possessed an anti-hydrolytic effect and this phenomenon was increased by increasing their concentration. Also, one has to point out that the use of the latter two fractions at 400 ppm level significantly exhibited anti-hydrolytic activity and were superior to that of BHT in retarding sunflower oil hydrolytic rancidity.

Figure (2) shows the changes in the peroxide value of sunflower oil mixed with phenolic fractions obtained for both leaves and fruits of Kronakii olive cultivar during storage. The data illustrated that the phenolic substances added to sunflower oil induced features of an autocatalytic chain reaction, *i.e.*, the rate of hydroperoxide formation increased with time. Since the curves in Fig. (2) show an increase in the peroxide value with time, the peroxide values at 14^{th} day storage period for all systems were divided by the peroxide value of the control (sunflower oil without any additives) to demonstrate the effect of the added materials on the stability of sunflower oil. Therefore, a value greater than one indicates a pro-oxidant effect while the values lower than one demonstrates an anti-oxidant activity. Sunflower oil without any phenolic components and mixed with BHT (200 ppm) systems was used as a guide to indicate the anti-oxidant or pro-oxidant activity. Accordingly, the relative peroxide values for BHT (200ppm), total (100, 200, 400 ppm) and free (100, 200, 400 ppm) phenolic componds of Kronakii leaves were 0.26; 0.40, 0.26, 0.12 and 0.40, 0.26, 0.12, respectively. Also, the relative peroxide values for esterified (100, 200, 400 ppm) and residual (100, 200, 400 ppm) phenolic compounds of Kronakii leaves were all the same (1.0). In addition, the relative values for all systems using phenolic compounds from fruits were calculated as mentioned before and the values were nearly similar to those obtained from leaves of Kronakii olive cultivar.

The antioxidant values for the systems containing 200 ppm of total and free phenolic compounds obtained from Kronakii leaves and fruits exhibited antioxidant activity similar to a system comprised of sunflower oil and BHT (200 ppm). On the other hand, the addition of esterified and residual phenolic compounds to sunflower oil of both leaves and fruits at various concentrations (100, 200, 400 ppm) did not exhibit any antioxidant activity on sunflower oil. It is worth mentioning that 400 ppm level of total and free phenolic compounds

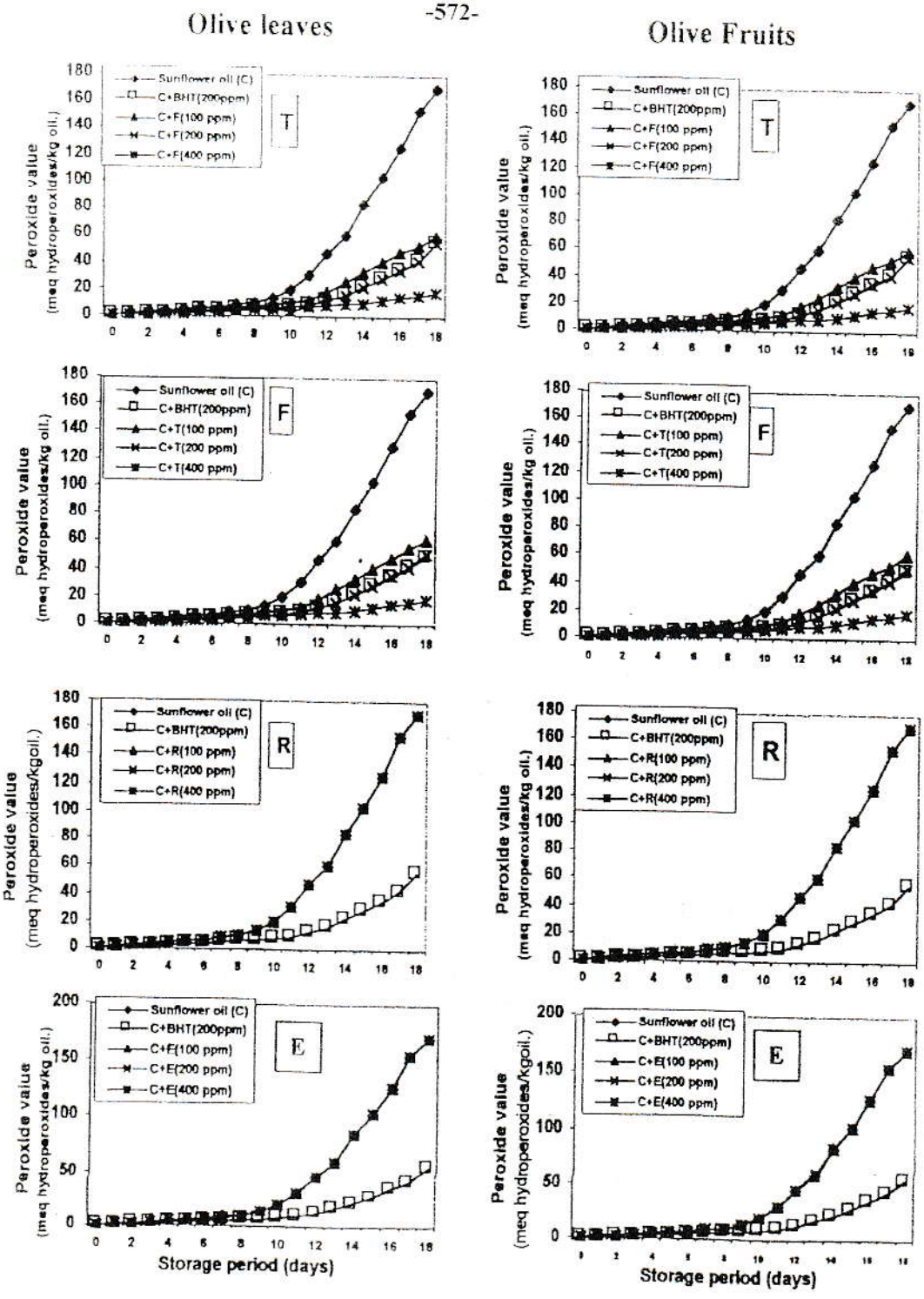

Fig.(2): Effect of total (T), free (F), residual (R) and esterified (E) polyphenolic compounds of Kronakii olivè fruits and leaves on the peroxide value of sunflower oil.

obtained from both leaves and fruits produced superior antioxidant power compared to that produced by BHT.

Fig (3) shows the TBA values for the systems consisted of sunflower oil (control), sunflower oil containing BHT (200 ppm), total (100, 200, 400 ppm), free (100, 200, 400 ppm), esterified (100, 200, 400 ppm) and residual, (100, 200, 400 ppm) phenolic compounds extracted from leaves and fruits of Kronakii olive cultivar. The results for TBA test indicated that the levels of secondary oxidation products from sunflower oil were very low and gradually increased with storage. The addition of BHT and total or free phenolic fractions obtained from both Kronakii leaves and fruits to sunflower oil significantly decreased the formation of secondary oxidation products at all concentrations. The formation of secondary oxidation products decreased by increasing the level of free and total phenolic fractions added to sunflower oil. The content of secondary oxidation products at the 16^{th} day of storage period for sunflower oil containing total (100, 200, 400 ppm) and free (100, 200, 400 ppm) phenolic compounds extracted from Kronakii leaves were 0.36, 0.21, 0.07 and 0.37, 0.23, 0.07, respectively. The respective values for TBA reacting species using the fruit phenolic compounds were 0.36, 0.21, 0.09 and 0.36, 0.21, 0.07, respectively. These data show that phenolic fractions of both leaves and fruits induced similar lowering effect on the formation of TBA reacting substances. On the other hand, the esterified and residual fractions of both fruits and leaves did not cause any significant effect towards decreasing the level of secondary oxidation products.

Several authors extracted various phenolic compounds from different plant sources and generally these compounds induced an increase in the shelf-life of some vegetable oils. For instance, polyphenols were extracted by Fayad *et al.*, (1989) from the olive oil using hexane, acetone and ethanol in a simple sequential procedure yielding three fractions (A, B, C). Fractions B and C were found to contain the highest ortho-diphenol concentration (about 3%). The addition of purified fraction B at a level of 100 ppm to refined olive or soybean oils partially inhibited the oxidative deterioration when the oils were stored in the dark at 100 °C. Also, Xing and White (1997) reported that the antioxidant activities of oat groats and hulls increased with the increased concentration. During 20 days of storage, the groat

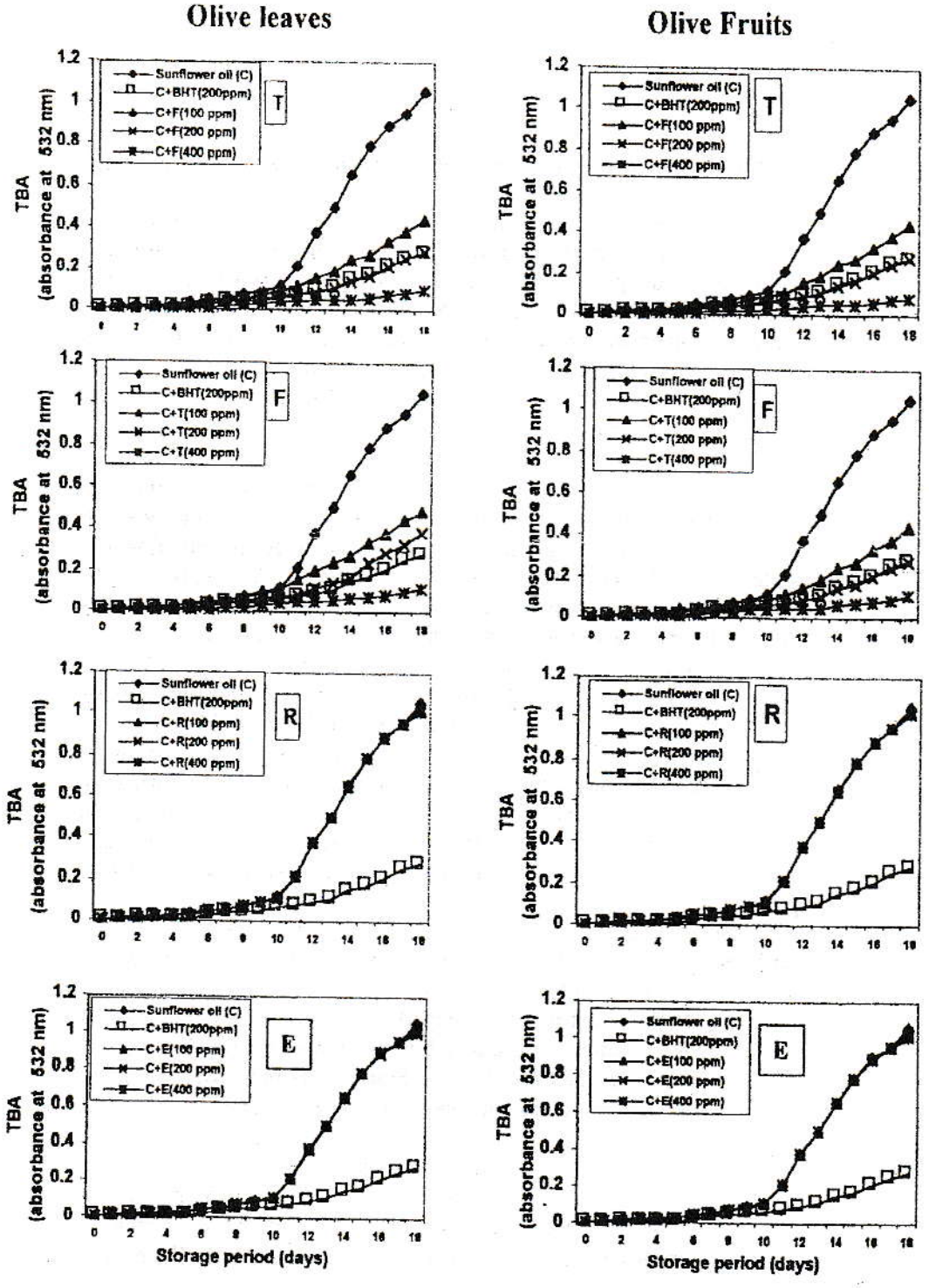

Fig.(3): Effect of total (T), free (F), residual (R) and esterified (E) polyphenolic compounds of Kronakii olive fruits and leaves on the thiobarbituric acid value (TBA) of sunflower oil.

extract (0.3%) was not significantly different from TBHQ after day 16, and hull extracts (0.2 and 0.3%) were not significantly different from TBHQ on day 20.

The main structure feature required for antioxidant activity is a phenolic ring containing hydroxyl group. This structural requirement is supported by the powerful antioxiant activites of the well-known synthetic BHT and the natural antioxidant thymol (Farag,and El Khawas., 1989 and Topallar *et al.*, 1997). One would relate the antioxidant activity to BHT or thymol to the inhibition of hydroperoxide formation. The first step in lipid oxidation is the abstraction of hydrogen atom from a fatty acid and oxygen involvement gives a peroxy radical. Generally, the antioxidants suppress the hydrogen atom abstraction from a fatty acid mocity which leads to the decrease of hydroperoxide formation. It is well known that the phenolic compounds act as hydrogen donors to that reaction mixture and therefore, the formation of hydroperoxides is decreased. The results of the present study are in line with these statements. Generally speaking the phenolic OH group has to be in the free form and if these groups are attached to other groups (such as glycosidic residue), it would prevent their antioxidant power due to the lack of hydrogen atoms donated to fatty acid radicals. This hypothesis is supported by the fact that the total and free phenolic compounds induced powerful antioxidant effect while, the esterified and residual phenolic compounds exhibited very little effect on retarding sunflower oil oxidative rancidity.

4.REFERENCES

Allen J.C. and Hamilton. R. J. (1983).Rancidity in Food., pp 85-173. London and New York. Applied Science Publishers.

A.O.C.S. (1985). Official and Tentative Methods of the American Oil Chemists Society, 3rd ed. American Oil Chemists Society, Champaign, IL.

Buck D. F. and Edwards M. K. (1997). Antioxidants to prolong shelf-life. Food Technology International, 29:33-37.

Charai M., Faid M. and Chaouch A. (1999). Essential oils from aromatic plants (*Thymus broussonetti Boiss.,Origanum compactum*

Benth., and *Citrus limon* (L.) N. L. Burm.) as natural antioxidants for olive oil. J. Essen. Oil Res., 11 : 517-521.

Dabrowski K. J. and Sosulski F. W. (1984). Composition of free and hydrolyzable phenolic acids in defatted flours of ten oilseeds. J. Agric Food Chem. 32: 128-130.

Dapkevicius A., Venskutonis R. , Beek T. A. and Linssen J. P. H. (1998). Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. J. Sci. Food. Agric., 77 :140-146.

Farag R. S., Abu-Raiia S. H. , El-Desoy G. E. and El-Baroty G. S. A. (1991). Safety evaluation of thyme and clove essential oils as natural antioxidants. African J. of Agricultural Sciences, 18:168-176.

Farag R. S., Ali M. N. and Taha S. H. (1990). Use of some essential oils as natural preservatives for butter. J. Am. Oil Chem. Soc. 68: 188-191.

Farag R. S. and El-Khawas K. H. M. M. (1989). Influence of γ-irradiation and microwaves on the antioxidant property of some essential oils. Ibid, 49:109-115.

Farag R. S., Hassan M. N. A. and Ali F. M.(1993). Beeswax and its unsaponifiable components as natural preservatives for butter and cottonseed oils. J. Food Sci. & Nutr., 44: 197-205.

Fayad Z., Sheabar A. and Neemann I. (1989). Separation and concentration of natural antioxidants from the rape of olives., 65:990-993.

Kanner J.,Edwin F., Rina G.,Bruce G. and John E. (1994). Natural antioxidants in grapes and wines. J. Agric. Food.Chem., 42, 64-69.

Namiki M. (1990). Antioxidants / antimutagens in food. Crit. Rev. Food Sci. Nutr., 29: 273-279.

Ottolenghi A.(1959).Interaction of ascorbic acid and mitochondrial lipids. Arch. Biochem. Biophys. 79:355-363.

Pratt D. E.(1992). Natural antioxidants from plant materials, in Phenolic compounds in food and their effect on health,. Ed. By M. T. Hung, C.T. Ho and C. Y. Lee, American Chemical Society, New York, PP. 55-71.

Steel R. G. D., and Torrie, J.H. (1980). Principles and procedures of Statistics, 3rd edn. McGraw-Hill, New York, U.S.A.

Topallar. H., Bayrak, Y. and Iscan, M. J. (1997). A kinetic study of the autooxidation of sunflower seed oil. J.Am. Oil Chem. Soc. 74: 1323-1327.

Tsimidou M. and Boskou D. (1994). Antioxidant activity of essential oils from the plants of the *Lamiacae* family. G. Charalambous (Ed) Spices, Herbs and Edible fungi.

Xinchu W., Goufeng C. , Xinwei D. and Shan D.(1998). Antioxidant activity of lizhicaa (*Salvia plebeia* R. Br.). J. Chinese Cereals and Oils Association. 13: 25-28.

Xing Y. and White P. (1997). Identification and function of antioxidants from oat groats and hulls. J. Am. Oil Chem. Soc. 74 :303-307.

تأثير مركبات الزيتون الفينولية على ثبات زيت عباد الشمس

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ملخص

تم استخلاص المركبات الفينولية من ثمار وأوراق الزيتون صنف كروناكى (Kronakii) وتم فصلها إلى ثلاثة أقسام هى: فينولات حرة و إسترات الفينولات و فينولات متبقية. تم خلط كل قسم من هذه الفينولات كل على حدة بالإضافة إلى الفينولات الكلية مع زيت عباد الشمس فى ثلاثة تركيزات مختلفة (١٠٠، ٢٠٠، ٤٠٠ جزء فى المليون) لدراسة طبيعتها من حيث قدرتها على خفض التحلل المائى وتأكسد زيت عباد الشمس. تم تقدير بعض ثوابت الزيت مثل رقم الحموضة – رقم البيروكسيد– رقم حمض الثيوباربتيوريك لزيت عباد الشمس منفرداً ثم مخلوطا بالفينولات المختلفة كمواد طبيعية مضادة للتزنخ والتخزين على درجة حرارة الغرفة. تمت مقارنة تأثير الفينولات المختلفة على ثبات الزيت مع تأثير مركب بيوتيلتيد هيدروكسى تولوين (BHT) كمادة مخلقة صناعيا مضادة للأكسدة.

أوضحت النتائج أن كل من المركبات الفينولية الكلية والحرة المستخلصة من ثمار وأوراق الزيتون صنف كروناكى كان لها تأثير واضح على خفض تحلل وتأكسد زيت عباد الشمس وزاد هذا التأثير بزيادة تركيز المركبات الفينولية. وعند تركيز ٤٠٠ جزء فى المليون المستخدم من الفينولات الكلية والحرة كان لها تأثير متميز على خفض التحلل وتأكسد زيت عباد الشمس يفوق تأثير BHT فى ثبات زيت عباد الشمس . والجدير بالذكر أن الفينولات الموجودة فى صورة إستر أو المتبقية لم تظهر أى تأثير مضاد لتزنخ زيت عباد الشمس.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٥٣) العدد الرابع (أكتوبر ٢٠٠٢):٥٦٥-٥٧٨.