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Contribution of α-tocopherol to olive oil stability

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The effect of 100, 500 and 1000 ppm of α-tocopherol on the oxidative stability of purified olive oil kept in the dark at 40°C was studied. Purified olive oil was prepared by liquid column chromatography and was practically devoid of minor constituents with possible pro-oxidant or antioxidant activity. α-Tocopherol acted as antioxidant at all levels of addition, although the antioxidant effect was greater at 100 ppm than at higher concentrations. In the initial stage of autoxidation a slightly pro-oxidant effect was observed. In the presence of strong antioxidants naturally occurring in olive oil, such as *ortho*-diphenols, α-tocopherol did not show any significant additional antioxidant effect during the period of low peroxide accumulation.

INTRODUCTION

The stability of virgin olive oil is related to the high levels of monounsaturated triacylglycerols and the presence of natural antioxidants.

α-Tocopherol is traditionally considered as the major antioxidant of olive oil (Sherwin, 1976; Bauernfeind, 1980). It comprises about 90% of the total tocopherols of this oil and its natural concentration varies between a few ppm up to 300 ppm (Sherwin, 1976; Bauernfeind, 1980; Coors, 1991). There is a plethora of publications on the antioxidant/pro-oxidant effect of α-tocopherol on lipids (Cort, 1974; Pongracz, 1984; Jung & Min, 1990) or emulsions (Cillard & Cillard, 1980; Cillard *et al.*, 1980*a*; Kruse & Eichner, 1993). This activity is dependent on both concentration (Cillard *et al.*, 1980*a,b*; Schuler, 1990) and temperature (Lee & Montag, 1992; Marinova & Yanishlieva, 1992). α-Tocopherol is a primary antioxidant for many substrates, although a pro-oxidant effect at different levels of addition has also been demonstrated (Cillard & Cillard, 1980; Jung & Min, 1990; Schuler, 1990). These discrepancies in the literature indicate that there is a need to investigate the role of α-tocopherol more thoroughly, especially in relation to the specific substrate used. This is important for olive oil, for which the relative bibliography is limited.

The addition of α- and γ-tocopherols in refined olive oil is beneficial for the stability of the oil (Juillet, 1975; Pongracz, 1984). However, with natural olive oil the problem is much more complicated because of the natural variability of tocopherol content and the presence of other pro-oxidant and antioxidant constituents.

Among the natural antioxidants present in olive oil, the most important class is polar phenolic compounds, such as 4-hydroxyphenylethanol (tyrosol), 3,4-dihydroxyphenylethanol (hydroxytyrosol), caffeic acid and other phenolic acids, as well as a number of unidentified esters and glycosides (Chimi *et al.*, 1988; Montedoro *et al.*, 1992; Tsimidou *et al.*, 1992). The level of total phenols varies from 25 to 440 ppm expressed as caffeic acid (Papadopoulos *et al.*, 1992; Fedeli & Cortesi, 1993). Among the polar phenolic antioxidants, *ortho*-diphenol compounds, e.g. hydroxytyrosol and caffeic acid, seem to have a pronounced activity. The activity of each phenol has been measured at various levels of addition (15–200 ppm) in refined olive oil (Chimi *et al.*, 1991; Nergiz, 1991; Papadopoulos & Boskou, 1991), but it is difficult to understand completely the contribution of each individual antioxidant to the overall stability of virgin olive oil.

The objective of this work is to study the role of α-tocopherol in olive oil and the extent to which the presence of polar phenolic antioxidants at realistic concentrations affects this activity. To eliminate other interferences, refined olive oil was purified through a chromatographic column, and the autoxidation of the purified triacylglycerols was monitored at 40°C by periodic measurements of peroxide values and absorbances at 232 nm.

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MATERIALS AND METHODS

Samples and standards

Refined olive oil was kindly donated by ELAIS S.A. (Athens, Greece). The standards for the oxidative stability tests were purchased from various suppliers: dl-α-tocopherol from Merck (Darmstadt, Germany); 3,4-dihydroxyphenylacetic acid from Sigma Co. (St Louis, MO, USA); tyrosol from Aldrich Chemical Co. (Milwaukee, WI, USA); and oleuropein from Extrasynthese (Z. I. Lyon-Nord, Genay, France).

Purification and chemical analyses of refined olive oil

Commercial refined olive oil was purified by a method proposed by Lampi *et al.* (1992) in the laboratory. The oil (200 g) diluted in *n*-hexane (200 ml) was passed through a chromatographic column (60 cm × 4 cm) packed in series with four different adsorbents suspended in *n*-hexane: 40 g activated silicic acid (Mallinckrodt Co., Paris, Kentucky, USA) (bottom layer); 20 g 1:2 mixture of Celite and activated charcoal (Riedel de Häen, Seelze, Germany); 80 g 1:2 mixture of Celite and powdered sugar (Merck, Darmstadt, Germany) and 40 g activated silicic acid (top layer). The eluent was collected until all the solution had been drawn into the column. The *n*-hexane was evaporated in a rotary evaporator at 40°C. Traces of the solvent were removed by flushing with nitrogen. The purified triacylglycerol fraction (100 g) was stored at −18°C. Peroxide value and acidity were determined according to IUPAC methods (IUPAC, 1987). To establish which pigments were present, the absorbances of the samples were measured at 430, 460, 550, 630, 670 and 710 nm. Total tocopherols expressed as α-tocopherol were determined by a colorimetric method with ferric chloride and 2,2'-dipyridine, applied to oils with no carotenes (Mahon & Chapman, 1954). The detection and removal of mono- and diacylglycerols during column chromatographic purification of refined olive oil was based on a RP-HPLC method (Tsimidou & Macrae, 1987).

Evaluation of stability

Purified refined olive oil samples containing α-tocopherol and/or other phenolic compounds at various concentrations were prepared. Antioxidants dissolved in ethanol were added. Ethanol was removed by an extended flushing with nitrogen and magnetic agitation of the sample at room temperature. The samples, 3 g each, were transferred to a series of open, transparent 8-ml glass bottles which measured 3 cm across, and the filled bottles were stored at 40°C in the dark. The rate of oxidation was followed by periodically measuring peroxide values and absorbances at 232 nm. Each parameter was measured twice. Seven determinations on a purified olive oil sample indicated good repeatability of peroxide value measurements ($\bar{x} \pm$ CV% = 3·5 ± 10), taking into consideration that the accuracy of the titration is 0·05 ml. The repeatability of the measurement of absorbance was also satisfactory (CV% = 3·5).

RESULTS AND DISCUSSION

Characteristics of purified refined olive oil

There is a need for standardization of methods to determine the oxidative stability of lipid systems. To eliminate the confounding effect of tocopherols and other natural antioxidants or pro-oxidant factors, evaluations need to be made with oil stripped of natural tocopherols and other minor constituents. All the experiments of this work were carried out with purified olive oil (POO) which was practically devoid of mono- and diacylglycerols and unsaponifiables.

Peroxide value, acidity, total tocopherol content and absorption in the visible region before and after purification are given in Table 1.

Effectiveness of α-tocopherol

The activity of α-tocopherol on the oxidative stability of POO was studied at 40°C because such a moderate temperature is very close to the conditions under which protection of olive oil against autoxidation is required. Other accelerated tests for determining lipid oxidation use higher temperatures, and the results obtained are very often poorly related to the rate of oxidation under normal conditions (Frankel, 1993).

α-Tocopherol at concentrations above 250 ppm has been reported to function as a pro-oxidant in purified soyabean oil (Young & Min, 1990). The experimental results of this work showed antioxidant activity of α-tocopherol at 100, 500 and 1000 ppm (Fig. 1); the lowest concentration was the most effective. An interesting irregularity was observed at the early stages of autoxidation, which is more clearly shown by the UV-measurements (Fig. 2). The stability of the control sample was greater than that of the oil samples containing α-tocopherol as additive for all the levels of addition. Similar observations on the activity of α-tocopherol in lard at ambient temperature (25°C) were reported by Marinova and Yanishlieva (1992). This trend may be

Table 1. Chemical characteristics of olive oil samples

	Refined olive oil	Purified olive oil
Peroxide value	7·5	0·3
Acidity value	0·08	0·04
Total tocopherols (ppm)	105	2
A_{430}	0·196	0
A_{460}	0·108	0
A_{550}	0·039	0
A_{630}	0·025	0
A_{670}	0·021	0
A_{710}	0·016	0

A, absorbance in the visible region.

due to the possible involvement of α-tocopherol in the propagation of the free radical formation from the lipid substrate. In order to reproduce the above phenomenon it was considered necessary to carry out a complementary experiment. POO samples of different degrees of autoxidation containing 100 ppm α-tocopherol were tested under the same experimental conditions. The results confirmed the pro-oxidant activity of α-tocopherol at the early stages of autoxidation (Fig. 3(a) and Table 2). The addition of 100 ppm α-tocopherol in moderately oxidised purified olive oil (15 meq. O_2/kg oil) had a significant antioxidant effect (Fig. 3(b)). These results are in accordance with a theoretical scheme presented by Schuler (1990):

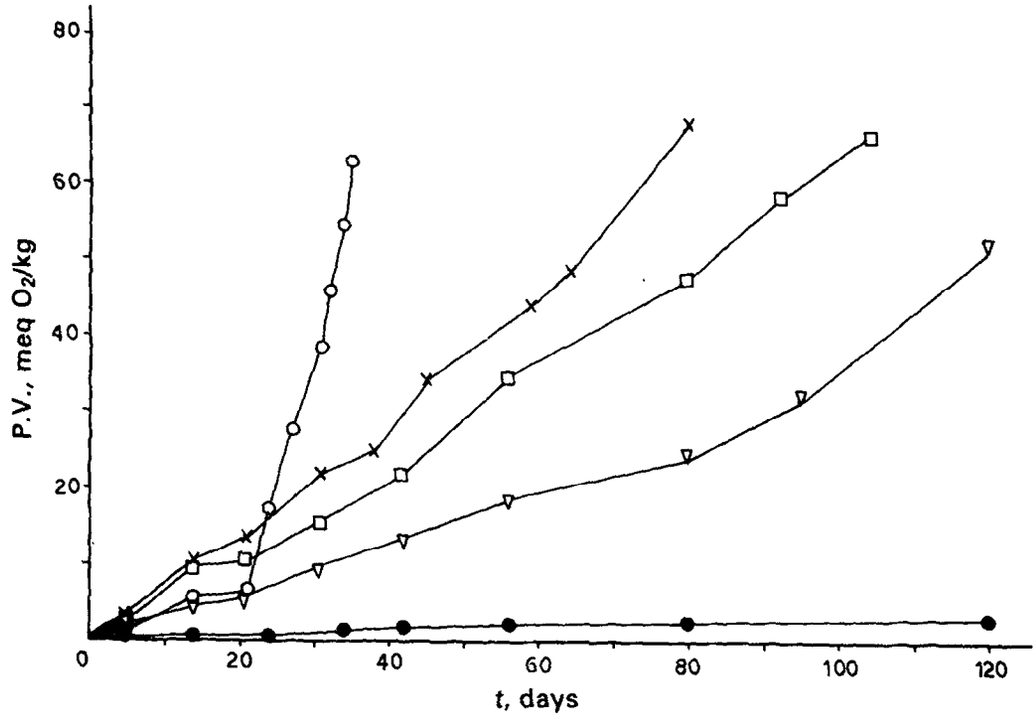

Fig. 1. Effect of α-tocopherol on the rate of peroxide formation in purified olive oil kept at 40°C. Control (○); plus BHT (●); plus α-tocopherol at 100 ppm (▽), 500 ppm (□), and 1000 ppm (×).

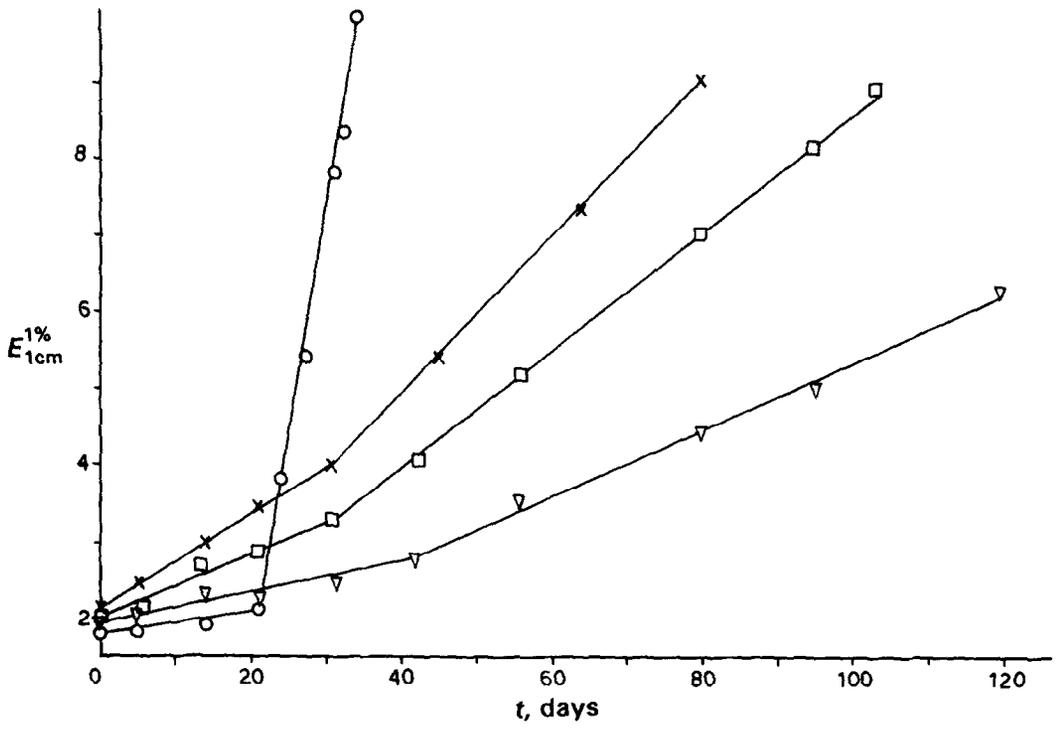

Fig. 2. Effect of α-tocopherol on the rate of conjugated diene formation on purified olive oil kept at 40°C. Control (○); α-tocopherol at 100 ppm (▽), 500 ppm (□), and 1000 pp, (×).

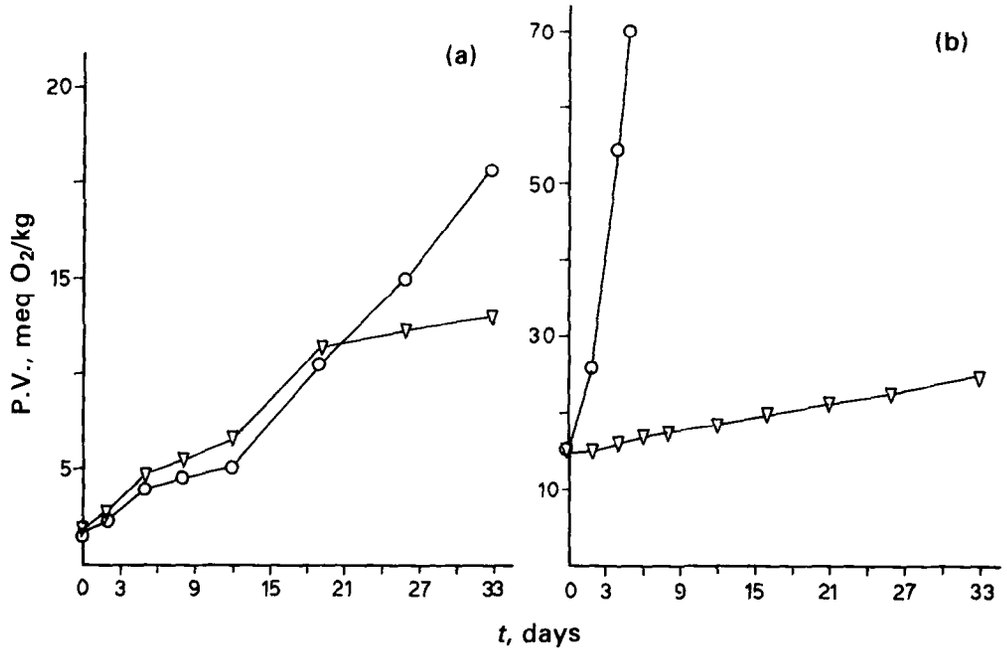

Fig. 3. Effect of α-tocopherol on the stability of purified olive oil kept at 40°C in relation to initial peroxide value (P.V.): (a) P.V.= 1.8 meq. O_2/kg POO; (b) P.V. = 14·3 meq. O_2/kg PO. Control (○); α-tocopherol at 100 ppm (▽).

Antioxidant activity: $ROO^{\cdot} + AH_2 \rightarrow ROOH + AH^{\cdot}$. Inactivation of radicals: $ROO^{\cdot} + AH^{\cdot} \rightarrow ROOH + A$ (oxidation); $ROO^{\cdot} + AH^{\cdot} \rightarrow ROOAH$ (addition); $AH^{\cdot} + AH^{\cdot} \rightarrow AHAH$ (dimerization); $AH^{\cdot} + AH^{\cdot} \rightarrow AH_2 + A$ (dismutation).
Pro-oxidant activity: $ROOH + AH^{\cdot} \rightarrow ROO^{\cdot} + AH_2$.
Where: ROOH = hydroperoxides; $ROO^{\cdot}$ = peroxide radicals; AH_2 = antioxidant (e.g. α-tocopherol); $AH^{\cdot}$ = antioxidant radicals.

It is clear from the above sequence of reactions that α-tocopherol may act both as a free radical scavenger (antioxidant) and as a free radical propagator (pro-oxidant) depending on the hydroperoxide concentration; this was stressed by Cillard *et al.* (1980). The theoretical scheme seems to fit in the case of olive oil, which is rich in monounsaturated triacylglycerols and the formation of hydroperoxides is not so rapid.

Table 2. Effect of α-tocopherol on the stability of purified olive oil kept at 40°C

Storage time (days)	Peroxide values (mequiv. O_2/kg oil)	
	Purified olive oil+ α-tocopherol (ppm)	
	0	100
0	1·8	1·8
2	2·4	2·6
5	4·0	4·8
8	4·5	5·5
12	5·2	6·5
19	10·5	11·3
26	15·1	12·3
33	20·8	13·0

Effectiveness of α-tocopherol in the presence of polar phenolic antioxidants

Figure 4 shows the rate of autoxidation of POO at 40°C in the presence of 3,4-dihydroxyphenylacetic acid and a mixture of this acid with tyrosol and oleuropein or α-tocopherol. 3,4-Dihydroxyphenylacetic acid was used instead of hydroxytyrosol which is not commercially available. The two compounds have similar antioxidant activity on refined olive oil (Papadopoulos & Boskou, 1991). The *ortho*-diphenol was added at a concentration representing one of the highest values reported for hydroxytyrosol in virgin olive oil (Tsimidou *et al.*, 1992; Montedoro *et al.*, 1992). Where a mixture of phenolic compounds was used, the concentration of *ortho*-diphenols (mmol/kg) was kept at the same level. After eight months of storage at 40°C the peroxide value of the sample with α-tocopherol was double that of the peroxide value of the sample containing only 3,4-dihydroxyphenylacetic acid. This indicates that the presence of α-tocopherol has an adverse effect during the period of low peroxide accumulation. Similar results are reported by Marinova and Yanishlieva (1992) who studied the effect of α-tocopherol on the oxidation of lard at room temperature. In their kinetic curves of peroxide accumulation in the initial stage of oxidation (up to 25 meq. O_2/kg lard) a clear pro-oxidant effect of α-tocopherol is evident. In the presence of *ortho*-diphenol, tyrosol and oleuropein, a less pronounced inhibition of oxidation was observed. This is in agreement with data presented by Chimi *et al.* (1991) who demonstrated a significantly inferior antioxidant activity of tyrosol and glycoside.

In a previous work (Papadopoulos *et al.*, 1993) stability of virgin olive oil was correlated to fatty acid

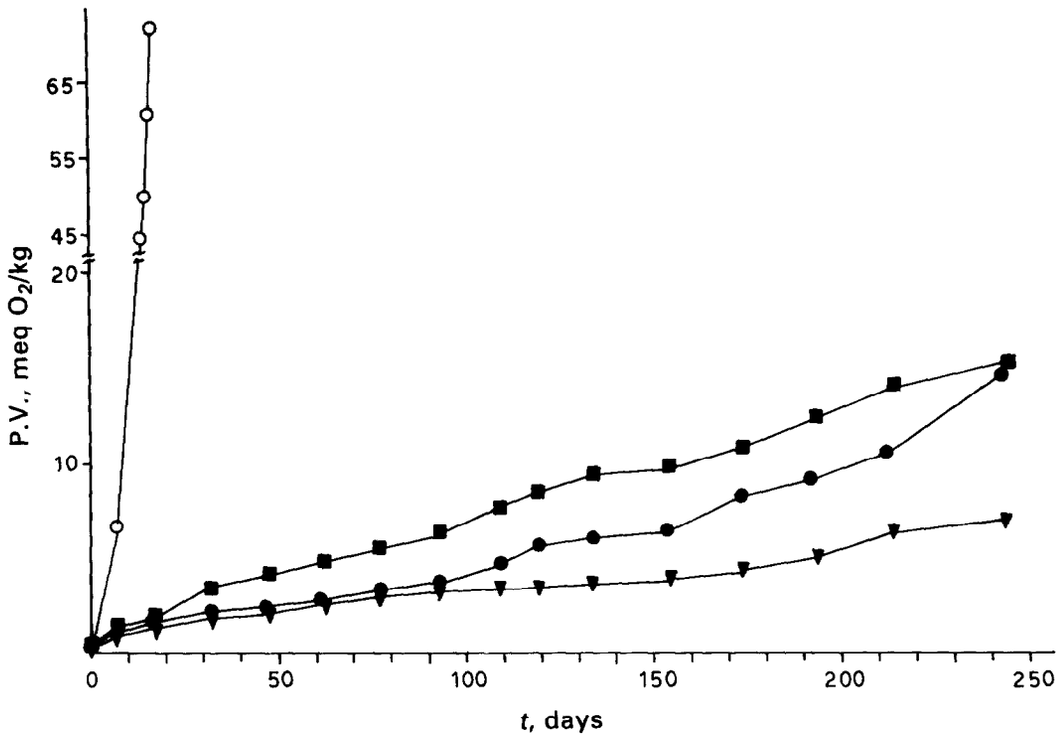

Fig. 4. Effect of polar phenolic compounds on the stability of purified olive oil kept at 40°C. Control (○); plus 40 ppm 3,4-dihydroxyphenylacetic acid (▼); plus 20 ppm 3,4-dihydroxyphenylacetic acid, 40 ppm tyrosol and 65 ppm oleuropein (●); plus 40 ppm 3,4-dihydroxyphenylacetic acid and 100 ppm α-tocopherol (■).

composition, polar phenolic compounds and tocopherol content, and quality indices such as acidity, peroxide value, anisidine value and spectrophotometric constants. By applying multivariate statistical techniques it was found that the oil stability depends on both the tocopherol and polar phenolic compound contents. These findings agree with experimental results obtained in this work. It appears that polar phenolic compounds are more important for the inhibition of autoxidation in the initial stages while α-tocopherol becomes effective when primary product of autoxidation reach a critical concentration. This observation may be significant for the commercial value of virgin olive oil. Already established quality criteria such as peroxide value are important in assessing olive oil stability, but to predict its shelf-life a better understanding is needed of the mechanism by which naturally antioxidant and pro-oxidant factors affect stability and relate to each other.

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