On the Role of Squalene in Olive Oil Stability

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The role of squalene in olive oil stability was studied for various concentrations and experimental conditions. No effect was found in induction periods of olive oil at elevated temperatures using the Rancimat apparatus. Samples were then stored at 40 and 62 °C in the dark, and the extent of oxidation was followed by periodic measurements of peroxide value and conjugated dienes. A concentration dependent moderate antioxidant activity was evidenced which was stronger in the case of olive oil compared to that found for sunflower oil and lard. In the presence of α-tocopherol (100 mg/kg) and caffeic acid (10 mg/kg) the contribution of squalene (7000 mg/kg) was not significant. No radical scavenging activity was observed using DPPH$^{\bullet}$ in 2-propanol. The weak antioxidant activity of squalene in olive oil may be explained by competitive oxidation of the different lipids present which leads to a reduction of the oxidation rate. Squalene plays a rather confined role in olive oil stability even at low temperatures.

Keywords: *Squalene; virgin olive oil stability; autoxidation; DPPH$^{\bullet}$; α-tocopherol; caffeic acid*

INTRODUCTION

The appreciable oxidative stability of virgin olive oil is mainly due to its characteristic pattern of triacylglycerols (Boskou, 1996) and also to the presence of polar phenolic antioxidants (Fedeli and Cortesi, 1993; Papadopoulos et al., 1993). α-Tocopherol, though not in abundance in the oil (Coors, 1991), contributes positively to its stability (Blekas et al., 1995). Studies on the effect of other constituents of virgin olive oil to its shelf life are scarce. For example, the role of squalene ($C_{30}H_{50}$) has not been examined although it accounts for more than 50% of the nonsaponifiable fraction of the oil.

Squalene, an intermediate compound in the biosynthesis of sterols in plant and animal world, is the major olive oil hydrocarbon. It makes up more than 90% of the hydrocarbon fraction (Eisner et al., 1965; Bastić et al., 1978; Perrin, 1992; Lanzón et al., 1994) ranging from 200 to 7500 mg/kg oil (Perrin, 1992) or even higher (800−12000 mg/kg oil) (Lanzón et al., 1994). Squalene content depends on olive cultivar (De Leonardis et al., 1998; Manzi et al., 1998), oil extraction technology (Nergiz and Ünal, 1990), and it is dramatically reduced during the process of refining (Mariani et al., 1992; Lanzón et al., 1994).

Squalene is regarded as partially responsible for the beneficial effects of olive oil against certain cancers. Recent laboratory studies suggest that squalene possesses chemopreventive activity against some types of cancer (Rao et al., 1998; Smith et al., 1998). Very little is known for the contribution of squalene to the oxidative stability of olive oil or other edible oils and fats. Some preliminary data have been reported for its antioxidant activity on methyl oleate and linoleate concentrates at 63 °C (Gowind Rao and Achaya, 1968). Similar behavior on the heat stability of refined rapeseed oil and two model lipid systems at 170 °C for 10 h has been cited by Malecka (1991). Their observations based on the measurement at high levels of peroxide accumulation or the reduction of fatty acid levels seem not very helpful in autoxidation studies. In a recent study on the content of minor constituents of Italian olive oils, derived from olives of six cultivars and different degrees of ripeness (Manzi et al., 1998), it was found that squalene loss during storage of oil samples in the dark was greater than that of α-tocopherol. This was attributed to a possible regeneration of α-tocopherol from squalene implying thus an antioxidant activity of this highly unsaturated hydrocarbon. More attention has been paid on the *in vivo* squalene role as one of the major human skin lipids. Under sunlight exposure, it was found to be the first target lipid on human skin surface acting as an effective quencher of singlet oxygen (Kohno et al., 1992; Kohno et al., 1995).

Though there is a lot of discussion about the oxidation products of olefinic systems only a few data exist on squalene oxidation products. It is reported that conjugated dienes can give rise to polymeric products from an early stage in their oxidation, whereas dienes with one-methylene interrupted double bonds give rise to hydroperoxides initially and polymerize at an advanced stage of oxidation. Dienes with two-methylene interrupted double bonds, such as squalene, give rise only to hydroperoxides whose decomposition is associated with carbon−carbon bond scission (Farmer and Sutton, 1942; Scott, 1965). Bolland and Hughes (1949) studying squalene autoxidation at 55 °C deduced that for each oxidized molecule two oxygen molecules were consumed. They suggested the formation of a diperoxide in four steps (Scheme 1). From the sequence of reactions it is evident that the peroxy radical cyclizes more efficiently than it abstracts a hydrogen atom from another squalene molecule. The determining factor appears to be a steric one, i.e., the particular spacing of the double bonds must

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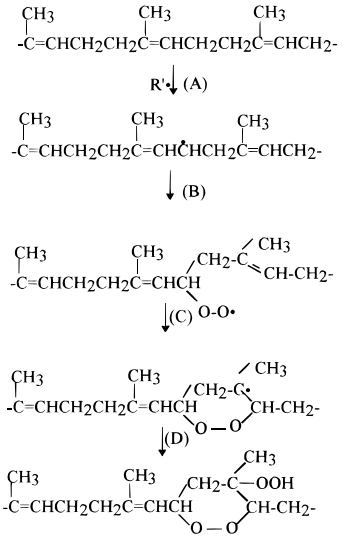
Scheme 1



be sufficiently favorable for cyclization of the peroxy radical. Additionally, it was mentioned that the products of squalene oxidation remain unchanged over a substantial range of oxygen uptake, so that they may not strongly participate in propagation reactions.

In this study the role of squalene in the autoxidation of purified olive oil was examined. The study was carried out for different concentrations of squalene and experimental conditions. Samples were stored at 40 and 62 °C in the dark, and the extent of oxidation was followed by periodic measurements of peroxide values and conjugated dienes. Assessment of antioxidant activity was also attained using induction period as determined by the Rancimat test at 100 and 120 °C. For comparison, squalene antioxidant activity was also examined for lipid substrates having different type and degree of unsaturation. The hypothesis made by Kohno et al. (1995) that squalene acts as a radical scavenger was examined through a reaction with a stable free radical. In all cases, its activity was compared with that of α-tocopherol and caffeic acid.

MATERIALS AND METHODS

Materials. Refined olive and sunflower oils were kindly donated by ELAIS S.A. (Piraeus, Greece). Lard was bought fresh from the market.

Solvents and Standards. Squalene and caffeic acid were from Sigma Co. (St. Louis, MO), and *dl*-α-tocopherol was from Merck (Darmstadt, Germany). Silicic acid (mesh size 100−200, Sigma, St. Louis, MO), Celite (Riedel de Häen, Seelze, Germany), commercial sucrose, activated carbon (<100 mesh, Aldrich, Dorset, England), and *n*-hexane (Riedel de Häen, Seelze, Germany) were used for column chromatography. Isooctane (spectranal), absolute ethanol, reagent grade trichloromethane, methanol, and acetic acid were from Riedel de Häen (Seelze, Germany). 2,2-Diphenyl-l-picrylhydrazyl (DPPH•) (approximately 90%) was from Sigma, (St. Louis, MO), and 2-propanol for spectroscopy was from Merck (Darmstadt, Germany).

Apparatus. A U-2000 Hitachi spectrophotometer (Tokyo, Japan) was used for the measurement of absorbance of the lipid substrate in the ultraviolet region and reduction of DPPH• absorbance at 515 nm. Induction periods of lipid substrates were measured using a Rancimat 617 apparatus (Metrohm, Herisau, Switzerland).

Purification and Chemical Analyses of Samples. The purification of refined olive and sunflower oils was carried out by a liquid chromatographic method (Lampi et al., 1992) adjusted for the requirements of the experiments. The oil (100 g/500 mL *n*-hexane) was passed through a chromatographic column (60 cm × 4 cm, i.d.) packed in series with four different adsorbents suspended in *n*-hexane. The four layers were as follows: 20 g of activated silicic acid and Celite mixture (1:1 w/w, bottom layer); 10 g of Celite and activated carbon mixture (1:2, w/w); 40 g of Celite and powdered commercial sucrose (1:2, w/w); and 20 g of activated silicic acid and Celite (1:1, w/w, top layer). The silicic acid was washed three times with distilled water and twice with methanol. Methanol was removed by evaporation, and finally the silicic acid activated at 120 °C for 10−12 h and at 200 °C for 12 h. The eluate was collected until all the oil solution had been drawn into the column. The *n*-hexane was evaporated in a rotary evaporator under vacuum at 40 °C. Traces of the solvent were removed by flashing nitrogen, and the purified oil was weighed and then stored at −18 °C. To remove the total α-tocopherol content from the refined sunflower oil, the eluate from the first column had to pass through a second one containing 10 g of 1:2 mixture of Celite and activated carbon suspended in *n*-hexane. Lard was melted at 80 °C and filtered through a common paper filter. The purification procedures were evaluated by determining the physicochemical characteristics of both the refined oils and the purified oils. Peroxide value and K_{232} and K_{270} (where $K_{\lambda max}$ = absorbance at λ_{max}/$C_{(g/100\ mL)}$) were determined according to the relevant IUPAC methods (IUPAC, 1987). The presence of pigments was monitored at 430, 460, 550, 630, and 670 nm. α-Tocopherol was measured by normal phase HPLC and UV detection at 294 nm (Psomiadou and Tsimidou, 1998). Total phenol content of the olive oil substrate was measured according to the Gutfinger (1981) procedure. Squalene levels in both refined and purified olive oil substrate were determined using a liquid chromatographic method (Cortesi et al., 1996). The fatty acid and triacylglycerol composition of all substrates was determined using official methods (EC, 1991).

Evaluation of Squalene Activity. *Oven Tests—Preparation of Model Systems for Evaluation of Antioxidant Activity*. The samples were prepared in conical flasks where the antioxidants were added dissolved in an appropriate solvent. The solvent was then evaporated by nitrogen flashing and magnetic agitation of the sample in the dark, at room temperature. A series of clear glass bottles (18 mm i.d.) of pharmacopoeia quality, containing 1 g of oil each, and a series containing 3 g of oil each were prepared for the measurement of peroxide value and for the measurement of absorbance at 232 nm, respectively. For peroxide value measurement the entire bottle content was used, while in the case of K_{232} an aliquot was taken from the bottle using a Pasteur pipet at appropriate time intervals. The ratio between sample amount and aliquot for the measurement of the absorbance at 232 nm was higher then 100. So, there was no effect on the autoxidation of the oil by the removal of aliquots. All analyses were performed in duplicate. The repeatability of peroxide value measurements was satisfactory: CV% = ± 3.5 for a mean value 7.5 mequiv O_2/kg and n = 7; CV% = ±10 for a mean value 0.3 mequiv O_2/kg and n = 7. The repeatability of K_{232} was CV% = ±2.3, n = 6.

Examination of Potential Antioxidant Activity of Squalene on Purified Olive Oil. Samples of purified olive oil containing different concentrations (200, 500, 1000, 2000, 4500, 12 000 mg/kg) of squalene were stored at 40 °C in the dark. Squalene was added dissolved in isooctane. Oxidation was monitored by measurements of peroxide value or absorption at 232 nm.

Evaluation of Squalene Antioxidant Effectiveness on Purified Olive Oil. Samples were stored at 40 and 62 °C, and oxidation was monitored as previously described. Induction periods were measured using the Rancimat apparatus at 100 and 120 °C.

Evaluation of the Effect of Lipid Substrate Unsaturation on Squalene Activity. The activity of squalene at two characteristic concentrations (200 and 7000 mg/kg) was also evaluated in purified sunflower oil and filtered lard by monitoring peroxide value and absorbance at 232 nm at 40 °C in the dark. The samples were prepared as described previously.

Evaluation of Squalene Activity in the Presence of Primary Antioxidants. α-Tocopherol and caffeic acid were added and dissolved in ethanol and methanol, respectively. The filled bottles were stored at 40 °C in the dark. The rate of oxidation was followed by periodic measurements of peroxide value.

Estimation of Potential Antiradical Activity of Squalene by DPPH•. The antiradical activity of squalene was determined using the free radical 2,2-diphenyl-l-picrylhydrazyl (DPPH•) in 2-propanol (6×10^{-5} M) based on procedures described by Brand-Williams et al. (1995) and Von Gadow et al. (1997). Monitoring of DPPH• reduction was by absorbance measurement at 515 nm. The exact initial DPPH• concentration in the reaction medium was calculated from a calibration curve using the equation $Abs_{515\ nm} = 10\,380 \times (C_{DPPH}) + 0.0066$ ($r = 0.999$). For comparison, the antiradical activity of α-tocopherol and caffeic acid was also tested. Different concentrations (expressed as number of moles of antioxidant/mole DPPH•) were used, and for each one the reaction kinetics was plotted. From these graphs, the percentage of DPPH• remaining at the steady state was determined, and the values were transferred onto another graph showing the percentage of residual stable radical at the steady state as a function of the molar ratio of antioxidant to DPPH•. The latter was used to determine the efficient concentration (EC_{50}) that is the amount of antioxidant necessary to decrease the initial DPPH• concentration by 50%. The lower the EC_{50} the higher the antioxidant activity. Moreover, the antiradical power (ARP) defined as $1/EC_{50}$ and the reaction time TEC_{50} needed to reach the steady state for EC_{50} (Sanchez-Moreno et al., 1998) were also calculated.

RESULTS AND DISCUSSION

Physicochemical Characteristics of Lipid Substrates. The fatty acid and triacylglycerol composition as well as the quality characteristics of the three lipid substrates are given in Table 1. The three substrates had different levels of oleic and linoleic acid for comparative examination of squalene activity. To eliminate the confounding effect of α-tocopherol and other natural antioxidants or pro-oxidant factors, the evaluation of squalene activity on the oxidative stability of the lipid substrates was carried out on the triacylglycerol fractions practically devoid of natural antioxidants and other minor constituents. Peroxide value, K_{232}, K_{270}, α-tocopherol content and absorption in the visible region, total phenol content, and squalene content of substrates were determined where this was necessary. As shown in Table 1, the purified oils and filtered lard were practically free of α-tocopherol and phenolic compounds having acceptably low initial peroxide values. The value of 4.1 mequiv O_2/kg for lard was much lower compared with initial values reported for its autoxidation studies by other investigators (Kim et al., 1994). The squalene content of refined olive oil is low due to the removal of squalene in the deodorizer distillate during refining. During refining isomerization products of squalene are also produced by the effect of temperature and bleaching earth during the bleaching process (Mariani et al., 1992; Lanzón et al., 1994). Indeed, in the refined olive oil used in this study, the concentration of squalene was much lower than the detection limit (3-fold the baseline noise) recorded for sensitivity of the refractive index detector set at 16×10^{-6} RIU (RI Detector Shimadzu, Tokyo, Japan). The detection limit was 200 mg/kg. Examination of the respective purified substrate showed a further reduction in the squalene content so that it was considered practically negligible for the requirements of the study. No squalene was detected in the other substrates examined.

Table 1. Fatty Acid, Triacylglycerol Composition, and Quality Characteristics of the Lipid Substrates[a]

fatty acid (%)	refined olive oil	refined sunflower oil	lard
C16:0	10.4	6.4	22.8
C18:0	0.8	4.2	11.6
C18:1	74.5	24.9	36.9
C18:2	9.7	61.9	20.5
C18:3	0.5	0.1	1.1
sum of other acids	4.1	2.5	7.1
triacylglycerols, (%) ECN			
50	13.3	5.3	20.8
48	48.5	13.8	33.2
46	23.8	24.0	30.1
44	14.3	33.3	12.6
42	–	23.6	3.4

quality characteristics	refined	purified	refined	purified	filtered
peroxide value (mequiv O_2/kg)	1.5	0	1.5	1.52	4.21
K_{232}, K_{270}	3.01, 0.98	1.93, 0.52	3.62, 1.62	2.95, 1.06	
α-tocopherol (mg/kg)	83.7	nd	238.4	nd	
total phenol content[b]	8	–	–	–	–
squalene (mg/kg oil)	<200	nd	nd	nd	nd
A_{430}	0.454	0	0.121	0	–
A_{460}	0.171	0	0.045	0	–
A_{550}	0.039	0	0	0	–
A_{630}	0.016	0	0	0	–
A_{670}	0.061	0	0	0	–

[a] ECN (equivalent carbon number) = CN (carbon number) − 2DB (double bond number). [b](mg caffeic acid/kg oil); A = absorbance in the visible region; [nd]= not detected; [–] = not measured as unnecessary.

Squalene Activity and Olive Oil Autoxidation. *Examination of Potential Antioxidant Activity of Squalene on Purified Olive Oil.* A stability test at 40 °C was undertaken for six different concentrations of squalene within the range reported for virgin olive oil by Perrin (1992). The results of periodic measurements of substrate absorbance at 232 nm are shown in Figure 1. It is evident that squalene had a protective effect on the oxidative stability of purified olive oil that was concentration dependent. This effect was then verified for another series of samples, containing 0, 2000, and 7000

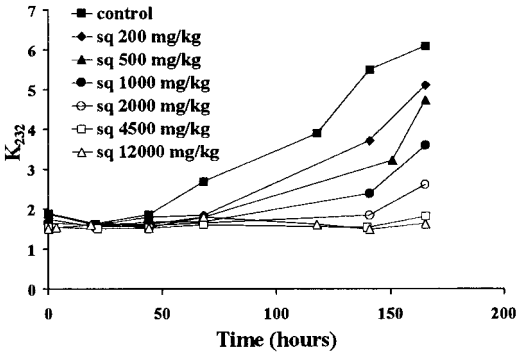


Figure 1. Kinetic curves of K_{232} increase during oxidation of purified olive oil at 40 °C in the presence of different concentrations of squalene; each point represents the mean of duplicate measurements.

mg/kg of squalene, at the same conditions of storage by monitoring the increase in peroxide value. The results are shown in Figure 2a. The finding that the protective effect of squalene increases with concentration is interesting taking into account that good quality virgin olive oils that satisfy the requirements set by European legislation (EC, 1991) usually contain high concentrations of squalene (800−12 000 mg/kg) (Lanzón et al., 1994). Subsequently, it was examined if the dependence on the level of squalene addition remained significant in the presence of primary antioxidants. α-Tocopherol was added at a concentration of 1000 mg/kg to ensure a more profound effectiveness at the early stages of oxidation. As shown in Figure 2b, in the presence of α-tocopherol the considerable increase in squalene content did not affect the rate of the early stages of oxidation.

Evaluation of Squalene Antioxidant Effectiveness on Purified Olive Oil. Adopting the suggestion made by Frankel (1993) for testing the effectiveness of potential antioxidants, different experimental conditions were used. The previous observations were verified for two extreme concentrations of squalene (200 and 7000 mg/kg oil) using (a) the Rancimat test at 100 and 120 °C and (b) the oven test at 40 and 62 °C.

(a) Rancimat Test. No antioxidant effect was observed using the Rancimat test in the purified olive oil for the two concentrations of squalene studied. The induction periods at both temperatures are presented in Table 2. Considering that the effect of squalene addition was not clear, possibly due to the high temperatures of the

Table 2. Effect of Squalene to the Induction Period of Purified Olive Oil as Measured by the Rancimat Test at 100 and 120 °C

	induction period[a] (h)	
sample	100 °C	120 °C
purified olive oil	2.6	0.9
+ 200 mg squalene/kg	2.6	0.9
+ 7000 mg squalene/kg	3.0	1.1

[a] Standard deviation= ±0.2 h, n = 6 measurements.

experimental procedure, oven tests at lower temperatures were then conducted.

(b) Oven Test. Figure 3 shows the process of peroxide and conjugated diene formation in purified olive oil kept at 40 (a, b) and 62 °C (c, d), respectively. Squalene indicated antioxidant activity for both temperatures at the concentration of 7000 mg/kg, while it appears to have almost no effect at 200 mg/kg. Protection factors (PF = T_1/T_2, where T_1 is the time required for the samples having the squalene to reach a peroxide value of 70 mequiv O_2/kg oil and T_2 was the respective time for the control) were not affected by temperature, i.e., 1.03 (1.01); 1.25 (1.23) for 200 and 7000 mg/kg addition of squalene, respectively, for the two temperatures. It is worth mentioning the overall slow kinetics of autoxidation of purified olive oil at 40 °C. The time required for the control to attain a peroxide value of 70 mequiv O_2/kg was five times greater than that required at 62 °C.

Effect of Lipid Substrate Unsaturation on Squalene Activity. To examine the squalene activity on lipid substrates with a different pattern of fatty acids and triacylglycerols compared with those of olive oil, sunflower oil, and lard were used (Table 1). Thus quite different induction periods were found for all the control samples. The behavior of squalene was also investigated for the same two extreme concentrations (200, 7000 mg/kg) at 40 °C. No antioxidant effect was observed on purified sunflower oil at both concentrations (PF is 1 and 1.03 for 200 and 7000 mg/kg, respectively). This behavior is illustrated in Figure 4a,b as monitored by measurement of hydroperoxide and conjugated diene formation.

In lard, a system rich in saturated fatty acids, the activity of squalene was similar to that evidenced for purified olive oil. Practically no effect was found in the case of 200 mg/kg squalene addition (PF = 1.05). A slightly lower protection factor with regards to that of

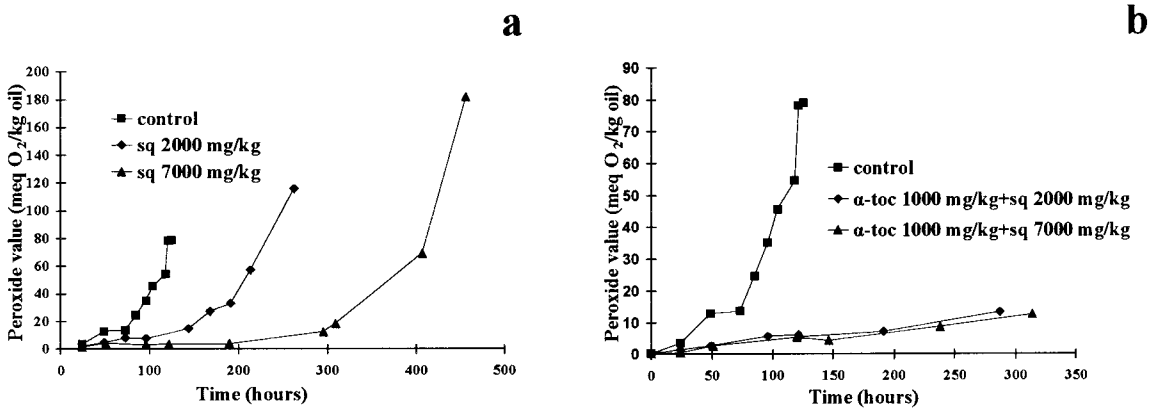


Figure 2. Kinetic curves of peroxide accumulation during oxidation of purified olive oil at 40 °C in the presence of different concentrations of squalene: (a) in the absence or (b) in the presence of α-tocopherol; each point represents the mean of duplicate measurements.

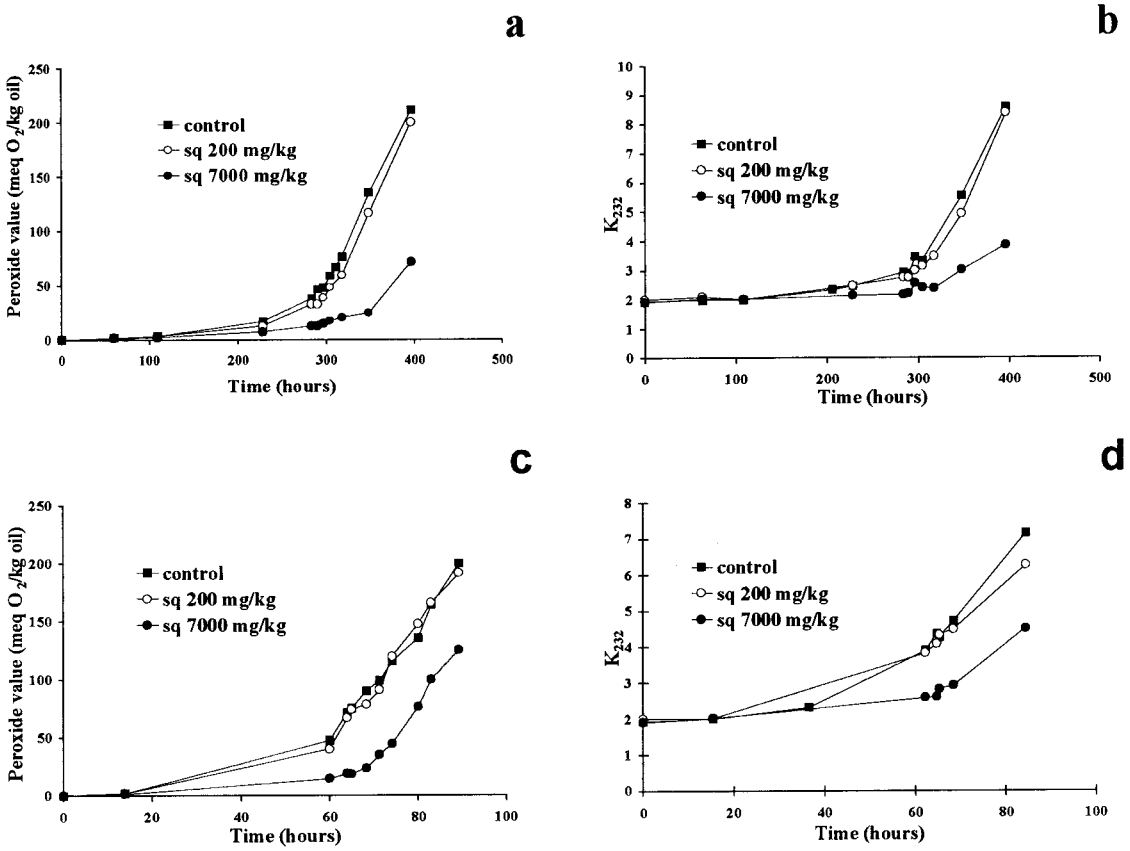


Figure 3. Effect of different concentrations of squalene on the peroxide value and K_{232} of purified olive oil kept at 40 °C (a, b) and 62 °C (c, d); each point represents the mean of duplicate measurements.

purified olive oil for the addition of 7000 mg/kg was noticed (PF = 1.14). The rate of initiation significantly affects antioxidant activity so that the degree of unsaturation of fats and oils greatly influences the relative efficacy of potential inhibitors (Kikugawa et al., 1987; Marinova and Yanishlieva, 1994). It has been found that for lard methyl esters half of the hydroperoxides formed during the initial stage of the process are of linoleate type and half of an oleate type, whereas linoleate hydroperoxides alone are formed during autoxidation of sunflower methyl esters (Yanishlieva and Popov, 1973). Linoleate is 10 times more readily oxidizable than is oleate (Gunstone and Hilditch, 1945), and the linoleate peroxide radicals are several times more reactive than are the oleate peroxide radicals (Yanishlieva et al., 1970). Oleate hydroperoxides are much more stable than are linoleate ones (Yanishlieva, 1973).

Squalene Activity in the Presence of Primary Antioxidants. The observation made during the preliminary work for the weak antioxidant activity of squalene in the presence of primary antioxidants was systematically examined. Two types of primary antioxidants were chosen. Caffeic acid was used instead of 3,4-dihydroxyphenylethyl alcohol (hydroxytyrosol) as a representative polar *o*-diphenol of virgin olive oil. Caffeic acid was added at a concentration naturally expected for total diphenol content of the oil (Gutfinger, 1981; Litridou et al., 1997). The major olive oil tocopherol at a characteristic concentration (100 mg/kg) was also used (Blekas et al., 1995). It was found that α-tocopherol becomes more effective when primary products of autoxidation reach a critical concentration, whereas the more polar phenolic antioxidants were consumed in the early stages of oxidation. Experiments were carried out for all possible combinations of squalene (7000 mg/kg), α-tocopherol (100 mg/kg), and caffeic acid (10 mg/kg). As it is shown in Figure 5, the overall duration of the experimental work was very long (ca. 2.5 months) compared with those reported for autoxidation studies (Marinova and Yanishlieva, 1994; Fuster et al., 1998) due to substrate properties and low storage temperature. Such a long incubation period was required in order the purified olive oil sample containing the most effective combination of antioxidants attained a peroxide value just above 20 mequiv O_2/kg. The latter has been set as the upper limit by the European Commission (EC, 1991) for edible virgin olive oils and is critical because above it the sensory characteristics of the oil are defective. As illustrated in Figure 5 squalene addition at 7000 mg/kg (B) was slightly effective compared to that of 100 mg/kg α-tocopherol (C) or 10 mg/kg caffeic acid (D). Curves C and E indicate that the presence of squalene does not affect the stability of olive oil in the range of low peroxide accumulation when α-tocopherol is present. These results verified the observations made in Figure 2b. Similar observations can be made from curves D and F concerning the effect of squalene in the presence of an *o*-diphenol, caffeic acid, known to be a more effective antioxidant for olive oil or in the presence of the mixture of the two primary antioxidants (G and H). Olive oil oxidative stability seems to be independent of the presence of squalene in the range of low hydroperoxide accumulation. This finding may be significant for the overall estimation of the nutrient value of virgin olive oil which has been recently also related to the chemopreventive properties of squalene (Rao et al., 1998; Smith et al., 1998).

Potential Antiradical Activity of Squalene by DPPH•.

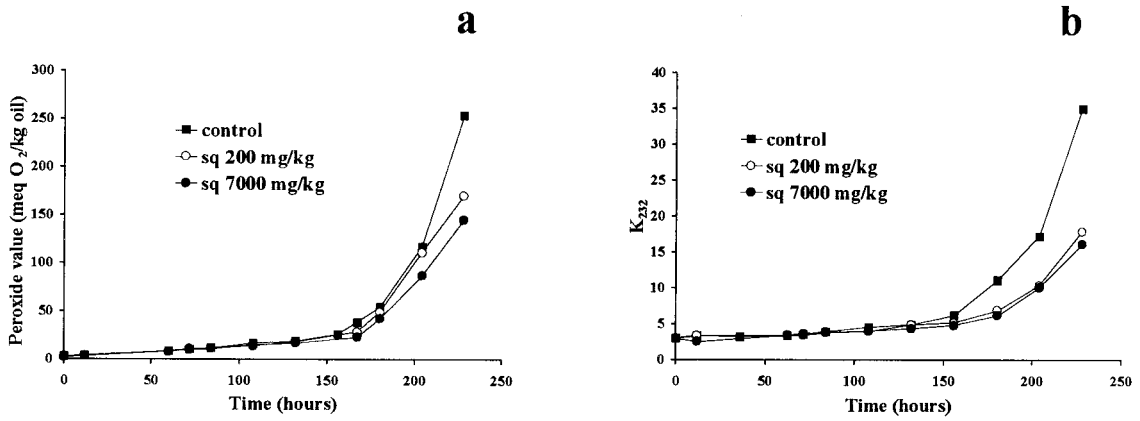


Figure 4. Effect of different concentrations of squalene on the peroxide value and K_{232} of purified sunflower oil kept at 40 °C; each point represents the mean of duplicate measurements.

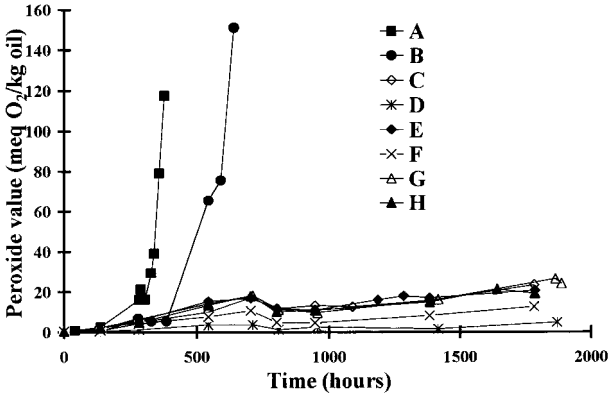


Figure 5. Effect of squalene on autoxidation of purified olive oil in the presence of α-tocopherol and caffeic acid at 40 °C, where A = control; B = + squalene, 7000 mg/kg; C = + α-tocopherol, 100 mg/kg; D = + caffeic acid, 10 mg/kg; E = + squalene, 7000 mg/kg + α-tocopherol, 100 mg/kg; F = + squalene, 7000 mg/kg + caffeic acid, 10 mg/kg; G = + α-tocopherol, 100 mg/kg + caffeic acid, 10 mg/kg; H = + squalene, 7000 mg/kg, + α-tocopherol, 100 mg/kg + caffeic acid, 10 mg/kg; each point represents the mean of duplicate measurements.

To understand the mechanism of the antioxidant effect of squalene on autoxidation of purified olive oil the chain breaking ability of squalene was examined. The reduction of DPPH• by squalene was monitored and compared with the respective behavior of α-tocopherol and caffeic acid. Results from the kinetic studies for the three compounds tested are given in Figure 6 and in Table 3. As can be seen from the type of curves and the EC_{50}, ARP, and T_{EC50} values squalene reacts very poorly with DPPH•. This is in agreement with data reported from Kohno et al. (1995) for squalene antiradical activity. In that study the reaction of squalene with models of LOO• (lipid peroxy radical) in *n*-butanol has been examined. It was deduced that the participation of squalene in reactions of the type LOO• + SQ → LOOH + SQ• was not significant. Squalene is rather stable to attacks by LOO• and has no chain breaking activity.

As a matter of fact the participation of squalene in virgin olive oil autoxidation seems to be confined. Squalene, having six isolated double bonds in its 30 carbon atom chain, is oxidized in a competitive way to olive oil triacylglycerols exhibiting antioxidant effectiveness which was found to be concentration dependent. Similar ≪competitive oxidation≫ phenomena have been observed in the past during the addition of a small amount of a highly reactive substrate to a less unsaturated one. In such cases, the oxidation rate of the mixture was slowed (Labuza, 1971; Rosas Romero and Morton, 1975; Liu and Chen, 1998) as experienced in the present

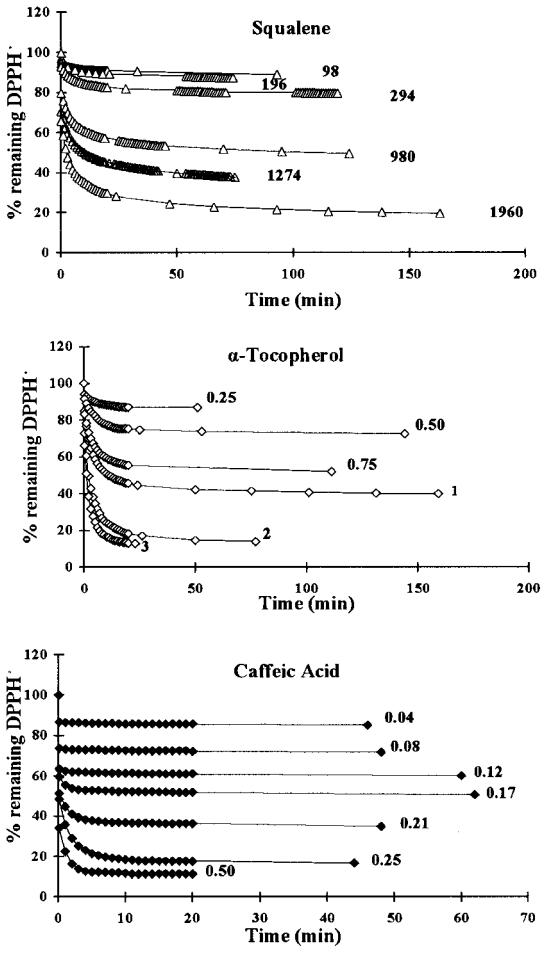


Figure 6. Kinetic behavior of squalene, α-tocopherol, and caffeic acid in 2-propanol (concentrations expressed as mole antioxidant/mole DPPH•).

Table 3. Calculation of EC_{50}, ARP, and T_{Ec50} Values According to the Kinetic Behavior for Squalene, α-Tocopherol, and Caffeic Acid during Reaction with DPPH• in 2-Propanol

compound	EC_{50} mole antioxidant/ mole DPPH	ARP	T_{EC50} (min)
squalene	980	0.001	≥70
α-tocopherol	0.22	4.55	13
caffeic acid	0.18	5.55	6

work for olive oil and lard. Additionally, the rather stable cyclic hydroperoxides expected to be formed from squalene may not easily participate in propagation reactions.

The presence of phenolic antioxidants with chain breaking and radical scavenging activities, such as caffeic acid or α-tocopherol, superimpose the above-mentioned mechanism and diminish the contribution of squalene to olive oil stability.

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