



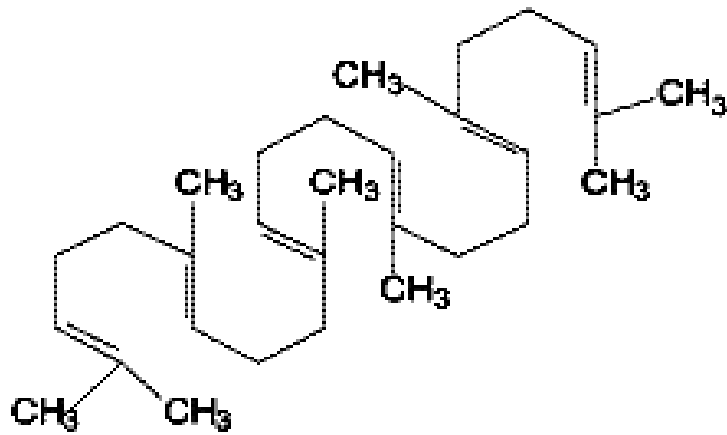
**Squalene**, a rather neglected  
constituent of virgin olive oil (VOO)  
*technological and nutritional aspects*

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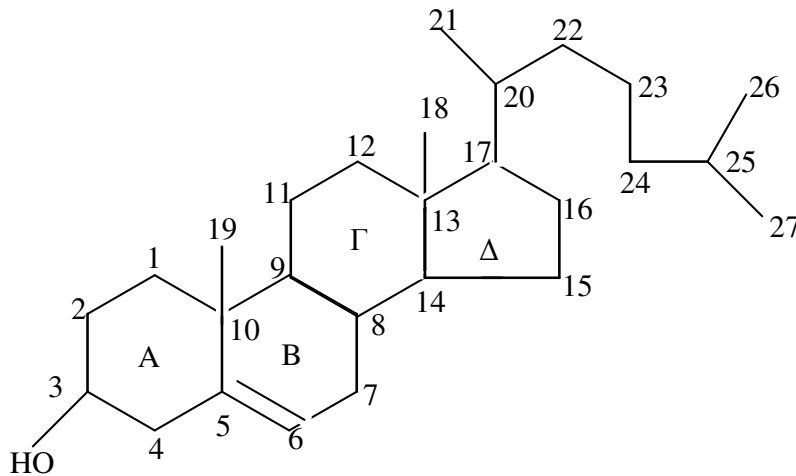
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# Squalene, SQ

2,6,10,15,19,23-hexamethyl-  
2,6,10,14,18,22-tetracosahexaene,  
(C<sub>30</sub>H<sub>50</sub>), *all-trans*



- The important precursor in sterol biosynthetic routes, is a terpenoid hydrocarbon



# Squalene

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- was originally identified in the beginning of the 20th century by the Japanese chemist Mitsumaru Tsujimoto in certain deep sea shark-liver oils.
- Depending upon age and sex, SQ content may reach 80% of the liver oil of such species (Wetherbee and Nichols, 2000 and references therein).

High levels of SQ are also found in human skin lipids ( $\sim 500 \mu\text{g g}^{-1}$  dry weight) & adipose tissue ( $\sim 300 \mu\text{g g}^{-1}$ ).

- The unique composition of skin lipids attracted the interest of researchers, who hypothesized a particular functionality of SQ and other unusual lipids, e.g. exclusion of pathogens due to metabolic problems that lead to the survival of only compatible microorganisms (Nikolaides, 1974).
- Daily SQ excretion by human skin is a strong evidence for its *de novo* synthesis there. Its role as an effective quencher of skin lipid photo-oxidation is well established.

This may explain the early commercial interest in SQ and its saturated counterpart, squalane, and the wide array of applications in cosmetics (e.g. sunscreens, moisturizing crèmes) and dyes (Eyres *et al.*, 2002).

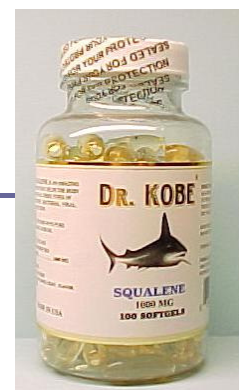
# Squalene in diet

- On the other hand dietary benefits through SQ consumption are not fully elucidated though is regarded to offer some protection against certain types of cancer ([Rao et al., 1998](#); [Smith, 2000](#)).
- Dietary SQ intake is reflected to triglyceride content increase more regularly than to plasma cholesterol levels ([Ostlund et al., 2002](#)).
- A hypothesis on the relationship of SQ and cancer risk-reducing effect of olive oil (suggested some years ago) had an impact on the renewed interest in this compound with reference to the Mediterranean Diet ([Newmark, 1997](#)).

## Daily SQ intake:

**USA:** 100 mg

**Greece:** 200 mg (annual olive oil consumption ~20 kg)



## Sources

- The excessive use of available marine sources led to a severe reduction of the population of certain sharks in the ocean
- so that alternative natural sources of SQ to be sought **in the plant kingdom.**
- The current interest in plant sources is more of ecological importance than of commercial potential

**taking into account that  
world SQ demand  
exceeds  
1000 tonnes sq per year**

# Squalene content is related to the lipid content of the plant material

This finding does not imply that all plant fats are rich in squalene.

There is a diversity in levels (0-12 g /kg oil)

- VOO prevails.
- Other fruit derived oils (palm oil, avocado oil) contain much lower levels.
- Among seed fats **corn oil** is far richer than soyabean, rapeseed or sunflower oils.
- Moreover, squalene is recovered from **the distillates of olive oil industry (10-30% yield)**, from *Amaranthus* seed oil (2-8 % w/w,) and other plant origin materials.



## Reports on the presence of squalene in VOO date more than fifty years back.

- The hydrocarbon fraction of VOO is made almost exclusively of SQ (Eisner *et al.*, 1965; Bastić *et al.*, 1978; Lanzón *et al.*, 1994)
- Ranges vary among reports (overall range 200-12000 mg / kg oil, **typical level ~ 5000 mg / kg**).
- The levels of SQ in VOO are related to cultivar characteristics (Guinda *et al.*, 1996; De Leonardis *et al.*, 1997; Manzi *et al.*, 1998)
- Extraction technology and refining process cause a considerable reduction in SQ levels (Mariani *et al.*, 1992; Lanzón *et al.*, 1994)
- Bleaching gives rise to the formation of isomers—3 % of C<sub>30</sub>H<sub>50</sub> content can be isomerized (Grob *et al.*, 1992) -, the determination of which is a criterion for the addition of bleached olive oil to VOO (Amelio *et al.*, 1998)

# Technological importance

- **Question 1:** Is SQ an endogenous olive oil antioxidant?
- **Question 2:** If yes, can be of use as an added antioxidant for other oils and fats, too?

# Radical scavenging activity ?

Despite the confusing data a weak activity is most probable (Psomiadou & Tsimidou, 1999).

Tab.1

Calculation of  $EC_{50}$ , ARP, and  $T_{EC50}$  Values

According to the Kinetic Behavior for Squalene,  $\alpha$ -Tocopherol, and Caffeic Acid during Reaction with DPPH $\cdot$  in 2-Propanol

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

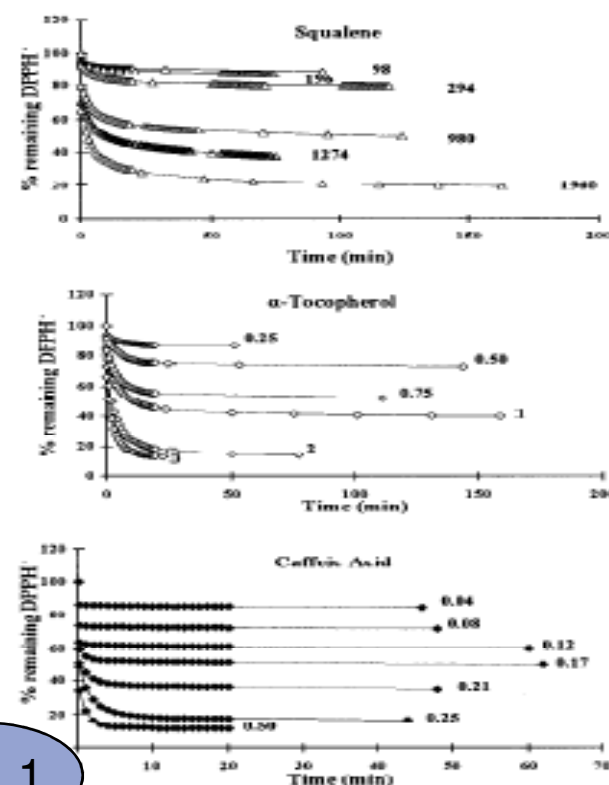


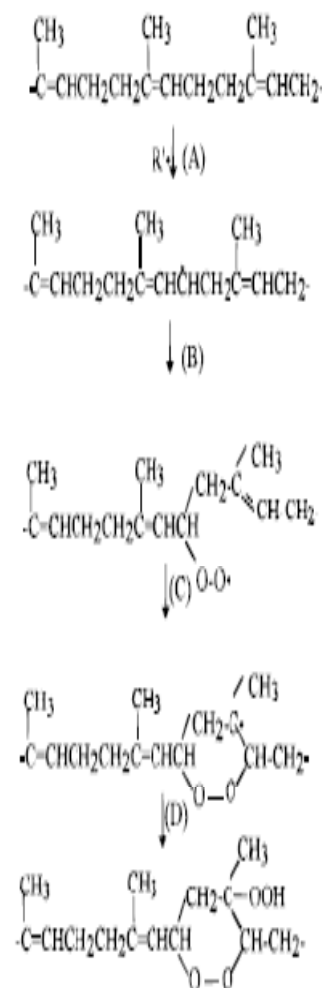
Fig. 1

6. Kinetic behavior of squalene,  $\alpha$ -tocopherol, and caffeic acid in 2 propanol (concentrations expressed as mole antioxidant/mole DPPH $\cdot$ ).

# 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 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.

Scheme 1





Contribution to VOO stability in  
the dark or under light exposure



Loss of squalene during VOO storage

## Effect on methyl oleate oxidation rate at 63° C (Rao & Achaya, 1968)

- SQ added was from the unasaponifiables of a specimen of olive oil, which contained 183 mg sq per 100 g oil.
- Increase in P.V was approximately linear for the first four days. During this period squalene showed a good protective action: in methyl oleate the P.V. increase per day was approximately 7 and 22 units in the presence and in the absence of 0.02 % sq
- Within this period, squalene had a better protective action than the same quantity of mixed tocopherols (from the unsaponifiables of safflower oil-90 mg/100 g of oil).
- In the subsequent storage period, while tocopherols continued to exert their protective effect, that of squalene was lost and the rate of P.V. increase became greater than that of the control.

The oxidation products of SQ may perhaps be pro-oxidant.  
Thus, SQ initially acts as an antioxidant  
but subsequently  
behaves as a pro-oxidant.

Loss of squalene during storage of VOO in closed glass bottles at temperature ranging from 10 to 20 °C in the dark for 6 months after production (Manzi *et al.*, 1998).

**Table 2**

Carotene,  $\alpha$ -tocopherol and squalene levels in the unsaponifiable fraction of oil samples extracted from different olive varieties: effect of 6 months storage (mean  $\pm$  SD,  $n = 3$ )

Cultivar	Carotene ( $\text{mg kg}^{-1}$ )			$\alpha$ -Tocopherol ( $\text{mg kg}^{-1}$ )			Squalene ( $\text{mg kg}^{-1}$ )		
	$t = 0$	$t = 6 \text{ months}$	Loss%	$t = 0$	$t = 6 \text{ months}$	Loss%	$t = 0$	$t = 6 \text{ months}$	Loss%
Gentile (Larino)I	2.12 $\pm$ 0.12	2.07 $\pm$ 0.04	2	169.41 $\pm$ 0.70	141.38 $\pm$ 0.31	17	3675.14 $\pm$ 6.95	2521.12 $\pm$ 35.37	31
Gentile (Larino)II	2.44 $\pm$ 0.46	2.37 $\pm$ 0.08	10	160.95 $\pm$ 1.10	118.88 $\pm$ 0.44	26	4028.92 $\pm$ 27.52	2448.57 $\pm$ 22.55	39
Gentile (Larino)III	1.68 $\pm$ 0.09	1.53 $\pm$ 0.06	9	178.71 $\pm$ 2.38	126.85 $\pm$ 0.30	29	4386.29 $\pm$ 10.64	2588.18 $\pm$ 11.73	41
Gentile (Colletorto)I	1.25 $\pm$ 0.13	1.25 $\pm$ 0.10	0	182.03 $\pm$ 1.80	155.90 $\pm$ 2.41	14	3428.65 $\pm$ 6.15	2544.79 $\pm$ 15.96	26
Gentile (Colletorto)II	2.42 $\pm$ 0.07	2.45 $\pm$ 0.15	0	206.76 $\pm$ 0.78	162.68 $\pm$ 1.84	21	4405.57 $\pm$ 35.15	2786.29 $\pm$ 15.22	37
Gentile (Colletorto)III	2.12 $\pm$ 0.07	2.09 $\pm$ 0.01	1	204.02 $\pm$ 0.38	156.09 $\pm$ 1.07	23	5099.24 $\pm$ 9.81	2906.87 $\pm$ 2.36	43
Coratina I	4.39 $\pm$ 0.04	3.98 $\pm$ 0.03	9	189.02 $\pm$ 1.95	159.39 $\pm$ 0.44	16	4504.39 $\pm$ 4.37	2869.38 $\pm$ 5.66	36
Coratina II	3.15 $\pm$ 0.07	2.98 $\pm$ 0.02	5	197.75 $\pm$ 0.50	161.91 $\pm$ 1.02	18	4454.10 $\pm$ 21.91	2763.21 $\pm$ 12.30	38
Coratina III	2.93 $\pm$ 0.27	2.87 $\pm$ 0.06	2	194.93 $\pm$ 4.05	158.10 $\pm$ 1.03	19	4649.28 $\pm$ 25.51	2775.59 $\pm$ 3.95	40
Peranzana I	3.39 $\pm$ 0.01	3.18 $\pm$ 0.19	6	239.25 $\pm$ 2.68	200.21 $\pm$ 2.89	16	5833.66 $\pm$ 59.22	3256.37 $\pm$ 49.35	44
Peranzana II	3.15 $\pm$ 0.16	3.10 $\pm$ 0.05	2	252.97 $\pm$ 0.39	197.42 $\pm$ 2.45	22	5772.25 $\pm$ 15.70	3066.02 $\pm$ 40.09	47
Rosciola	2.01 $\pm$ 0.11	1.89 $\pm$ 0.01	6	167.17 $\pm$ 3.31	135.63 $\pm$ 0.60	19	3944.94 $\pm$ 15.20	2584.56 $\pm$ 9.28	34
Leccino I	2.20 $\pm$ 0.19	2.18 $\pm$ 0.10	0	245.83 $\pm$ 0.07	187.64 $\pm$ 0.07	24	5018.58 $\pm$ 58.71	3113.86 $\pm$ 14.68	38
Leccino II	1.89 $\pm$ 0.11	1.90 $\pm$ 0.10	0	196.95 $\pm$ 2.96	133.97 $\pm$ 2.86	32	4593.20 $\pm$ 12.56	2745.84 $\pm$ 12.34	40
Olivastro	2.99 $\pm$ 0.09	2.93 $\pm$ 0.05	2	208.99 $\pm$ 3.31	159.77 $\pm$ 0.71	24	5885.33 $\pm$ 55.07	3251.01 $\pm$ 23.80	45

Tocopherol and SQ act as chain-breaking antioxidants. They scavenge the chain-carrying peroxy radicals, interrupt the chain propagation, but are themselves modified during this reaction. The former is reported to scavenge radicals faster than the latter, but the tocopheroxyl radical formed could be reduced by squalene to regenerate tocopherol.

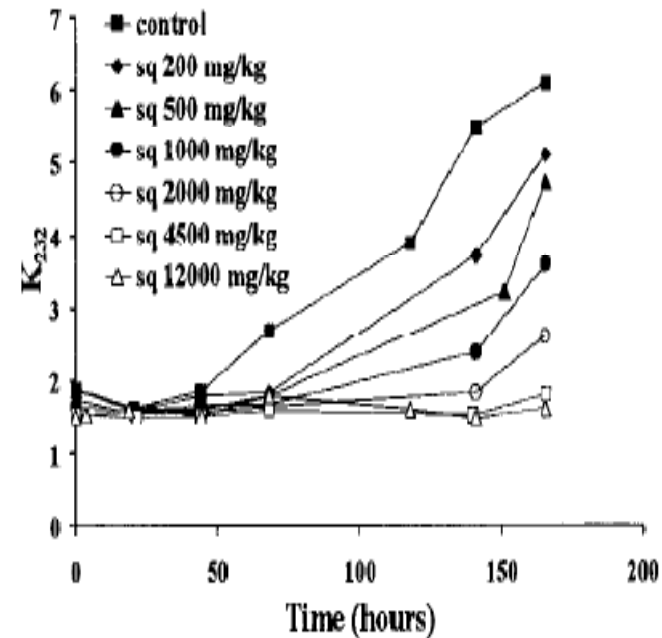
Thus, tocopherol is not consumed at first and only SQ disappears.

This hypothesis could explain why tocopherol and SQ loss is greater than carotene loss and why tocopherol loss is lower than SQ loss.



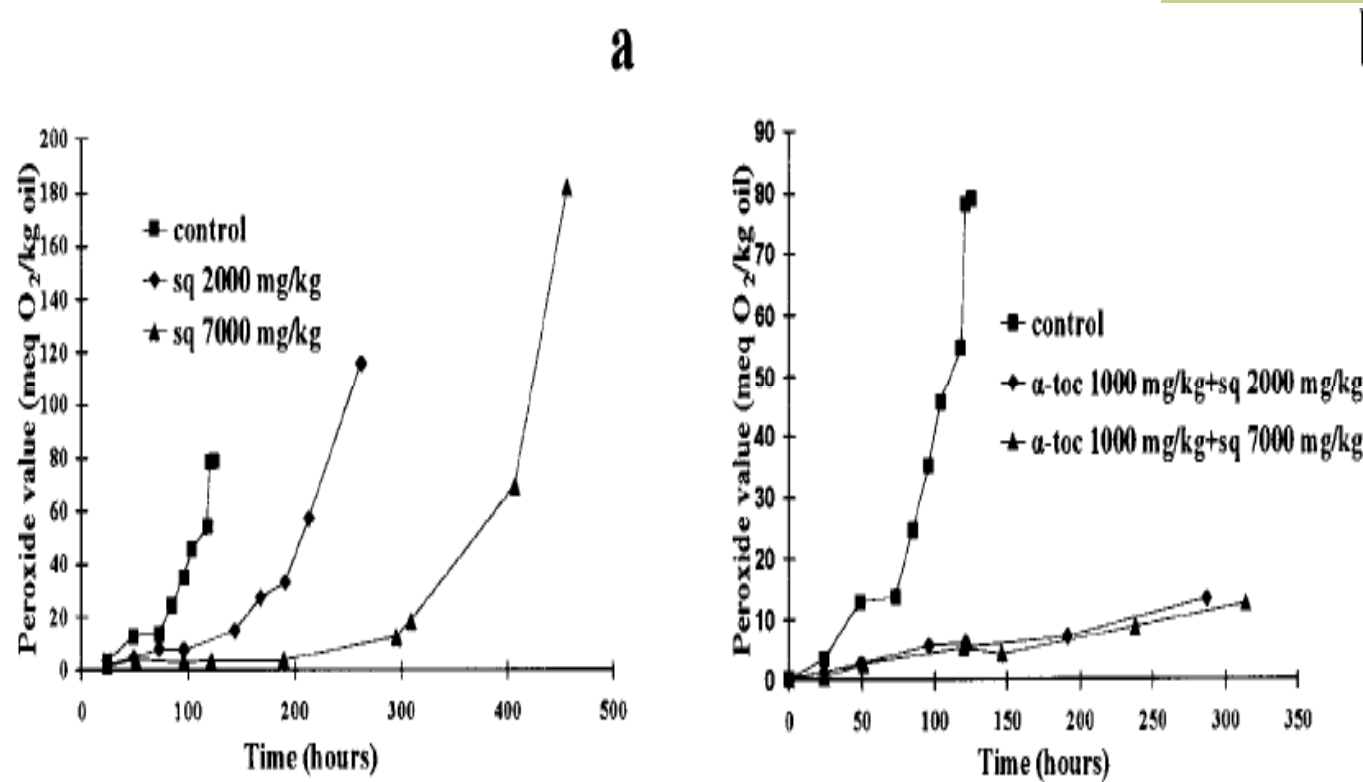
## Psomiadou & Tsimidou (1999)

- studied the role of SQ on the stability of OO (devoid of prooxidants/antioxidants) for various concentrations and experimental conditions.
- A concentration dependent moderate antioxidant activity was evidenced



**Fig.2** 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.

In the presence of  $\alpha$ -tocopherol the considerable increase in SQ content did not affect the rate of the early stages of oxidation at 40 °C



**Fig. 3** 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  $\alpha$ -tocopherol; each point represents the mean of duplicate measurements.

No effect was found in induction periods of olive oil at elevated temperatures using the Rancimat apparatus.

Tab. 3

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

sample	induction period <sup>a</sup> (h)	
	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

<sup>a</sup> Standard deviation=  $\pm 0.2$  h,  $n = 6$  measurements.

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. **SQ (7000 mg/Kg) indicated antioxidant activity at both temperatures**

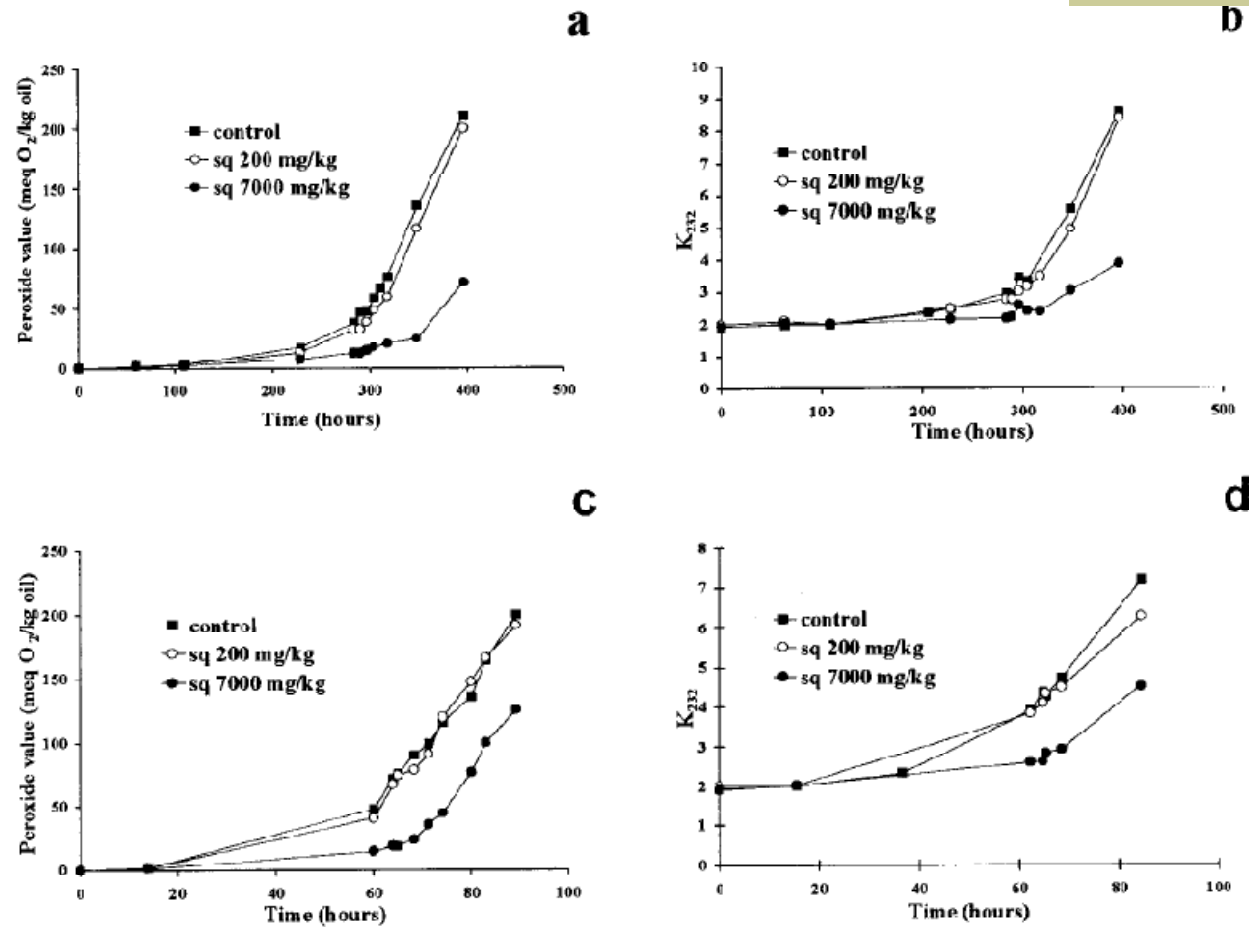
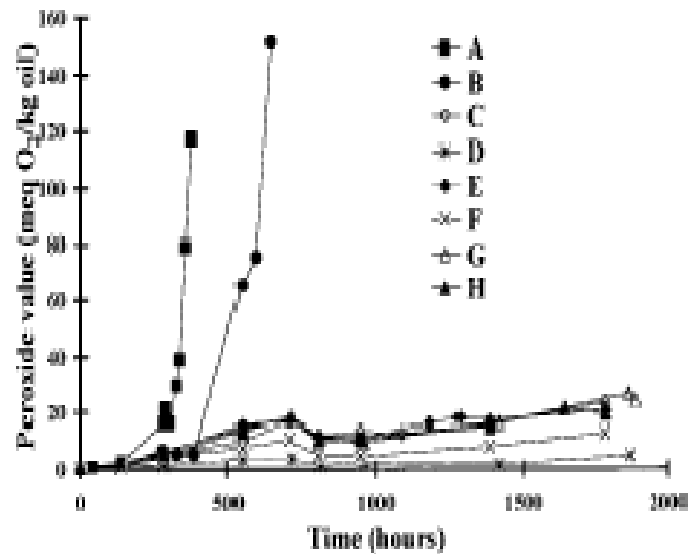


Fig.4

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.

In the presence of  $\alpha$ -tocopherol (100 mg/kg) and caffeic acid (10 mg/kg) the contribution of SQ (7000 mg/kg) was not significant.



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

As a matter of fact the participation of SQ in VOO autoxidation seems to be confined.

# Explanation?

- SQ, is oxidized in a competitive way to OO 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 lessunsaturated 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 work for OO and lard but not for sunflower oil.

Additionally, the rather stable cyclic hydroperoxides expected to be formed from squalene may not easily participate in propagation reactions.

## Psomiadou and Tsimidou (2002a) verified that confined role for real VOO samples

- Two series of bottles for each sample were stored in the dark at ambient temperature.
- One was opened periodically, and oil aliquots were withdrawn for analyses; the other remained closed throughout the storage period.
- Changes in the lipid substrate and the evolution of the minor components during storage were followed to obtain a better understanding of oil stability and the interactions that possibly take place among components.
- Measurement of changes in the content of SQ by HPLC in the present study revealed an insignificant decrease in all but one sample (10% loss).
- Their results were not in agreement with those by Manzi et al., 1998

SQ was rather stable at the experimental conditions despite the long storage period.

**Complementary experiments using standard compounds on OO stripped of antioxidants and pro-oxidants were also carried out to further investigate interactions**

- **SQ and  $\alpha$ -tocopherol were added separately and as a mixture**, and the samples were stored in the dark at 60 °C.
- Changes in the content of the two compounds as well as changes in the lipid substrate were measured.
- The mixture of  $\alpha$ -tocopherol and SQ > than  $\alpha$ -tocopherol alone.

No SQ loss was observed even in the presence of  $\alpha$ -tocopherol

1. One may assume that the presence of SQ did not protect  $\alpha$ -tocopherol during the long storage period.
2. SQ was practically stable during storage at 60 °C even for high PVs (>200 mequiv of O<sub>2</sub>/Kg). The same trend was observed in the samples containing the combination of  $\alpha$ -tocopherol and SQ.
3. These findings support the evidence obtained from the storage of VOO samples, confirming that SQ is quite stable during autoxidation.

Furthermore, it may be postulated that the products of SQ oxidation are quite stable and would not be further involved in propagation reactions.



(Mateos et al., 2003) investigated the possible synergistic effect of SQ with  $\alpha$ -tocopherol and hydroxytyrosol.

- HOSO (containing 653 mg of  $\alpha$ -tocopherol/kg), PHOSO (purified high oleic sunflower), and PHOSO spiked with 0.20 mmol of hydroxytyrosol/kg were fortified with SQ.

- No statistical differences were found between results within each set of samples, indicating a negligible effect of SQ on the stability of matrices with fatty acid composition similar to that of olive oils.

Tab. 4

Effect of Squalene on the Rancimat Stability of Purified High-Oleic Sunflower Oil (PHOSO), PHOSO Spiked with 0.2 mmol/kg of Hydroxytyrosol (HTy), and High-Oleic Sunflower Oil (HOSO) [Mean Values  $\pm$  SD ( $n = 4$ )]

squalene added (mg/kg)	induction time (h)		
	PHOSO	PHOSO + 0.2 mmol/kg of HTy	HOSO
0	2.06 $\pm$ 0.09	13.90 $\pm$ 0.66	22.0 $\pm$ 1.1
2000	2.09 $\pm$ 0.08	14.01 $\pm$ 0.63	20.5 $\pm$ 1.0
4000	2.19 $\pm$ 1.00	13.38 $\pm$ 0.69	22.3 $\pm$ 0.9
6000	2.04 $\pm$ 0.07	13.51 $\pm$ 0.58	21.5 $\pm$ 1.3
8000	2.10 $\pm$ 0.08	14.20 $\pm$ 0.61	21.9 $\pm$ 1.0

## (Hrnciric & Fritsche, 2005)

- The changes in minor components, related to the stage of ongoing oxidation and expressed as a percentage of the induction period (IP), followed a similar pattern in the examined oils:
- o-diphenols diminished by the highest rate (halved within 15% of the IP), followed by a-tocopherol (halved within 35% of the IP). Carotenoids and chlorophylls were also affected by autoxidation, whereas SQ showed high stability (<20% loss within 100% of the IP).

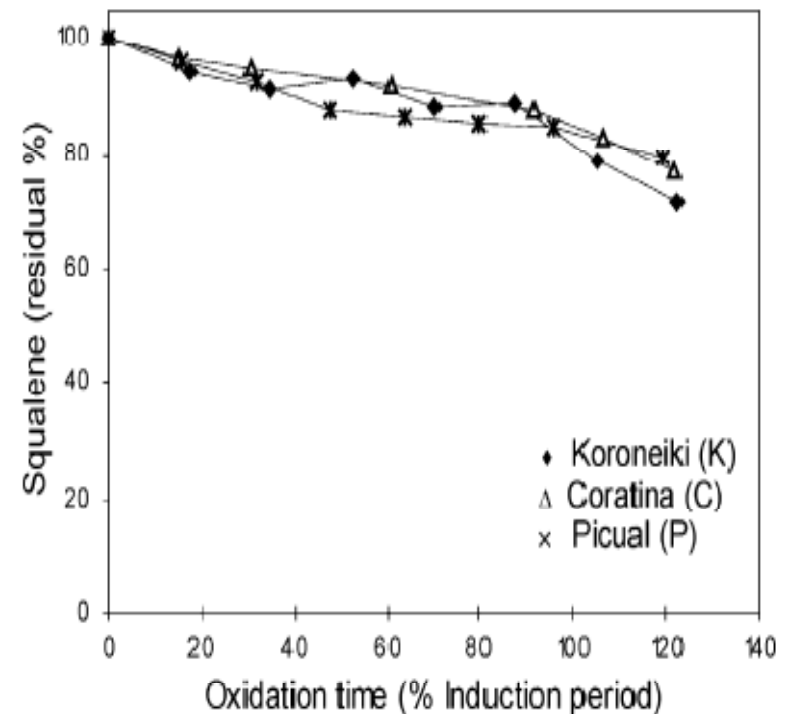


Figure 6. Changes in the residual content of squalene in extra virgin olive oils subjected to accelerated storage conditions at 60 °C. RSD < 7.5% of the mean values ( $n = 2$ ).

## Conforti *et al.*, 2005

- Lipid peroxidation of liposomes was followed using the TBA test.

- SQ showed a significant concentration dependent effect

- %Inh:

✓ 91% (0.1 mg/ml)

✓ 35% (0.01 mg/ml)

## Finotti *et al.*, 2000

- They investigated the possible synergistic effect of  $\alpha$ -tocopherol,  $\beta$ -sitosterol and SQ using the crocin bleaching inhibition assay.
- For this purpose each single compound and mixtures of them, with 2 and 3 components, were analyzed by the method at 40 °C.
- At the concentrations tested SQ showed a little antioxidant effect and on the contrary,  $\beta$ -sitosterol showed a pro-oxidant effect. No significant concentration effect was detected for SQ.
- All the mixtures tested showed a positive value, i.e. an antioxidant capacity. All the mixtures presented also a synergistic effect.

A positive explanation is that the SQ could act as a competitive compound, reducing the oxidation rate, even if it did not present significant antioxidant activity when tested alone.

# Squalene as a quencher of singlet oxygen

- The kinetic study of quenching reaction of singlet oxygen carried out by by [Kohno \*et al.\*, \(1995\)](#) showed that the rate constant of quenching of singlet oxygen (kQ) by SQ was >> than those of the lipids in human skin surface.
- Sq is the first target lipid in human skin surface by oxidative stresses such as sun light exposure, kQ of SQ is similar to that of 3,5-di-t-butyl-4-hydroxytoluene (BHT) and less than that of  $\alpha$ -tocopherol.
- The large kQ of SQ is due to the small ionization potential. Sq consists of six 2-methyl-2-pentene units and kQ of SQ is about 6-times as large as that of 2-methyl-2-pentene.
- The electron donating property of methyl groups bonded to quaternary carbons of SQ is essential to the large kQ. SQ is not very susceptible to peroxidation and is stable for attacks by peroxide radicals. The chain reaction of lipid peroxidation is unlikely to be propagated with SQ in human skin surface.

It is concluded that  
SQ functions as an efficient quencher of singlet oxygen  
and  
prevents the corresponding part of lipid peroxidation in human skin surface.

# Psomiadou & Tsimidou, 2002b

- $\beta$ -Carotene and lutein contents remained almost unchanged in the course of the photo-oxidation experiments. This supports the physical quenching mechanism through which they act as quenchers.
- More changes were found in the tocopherol content. Indeed, Tocopherol loss was higher than that observed for the total polar phenol content except for one sample.
- $\alpha$ -Tocopherol loss was gradual and increased with exposure time in contrast with total phenol content loss. The extent of reduction ranged from 20 to 35% and depended on the exposure period.

A small SQ loss (4- 12%) was observed in all samples after the exposure period.

## *EXPERIMENTAL:*

*Oil samples (10 mL) were poured into 15 mL transparent glass bottles (7% headspace).*

*The closed bottles were placed in the center of a metallic shelf in the light chamber.*

*The distance of the samples from the fluorescence lamps was 30 cm, and between the samples adequate space was left to ensure equalized exposure.*

*The temperature of the chamber was kept at 25 (  $\pm$  1  $^{\circ}$ C, and the light intensity at sample level was 12100 lx as measured by a pyranometer (model CM21, Kipp and Zonene, Delft, Holland).*

*Photooxidation was followed by measuring chlorophyll and Pheo a contents.*

# Psomiadou & Tsimidou, 2002b

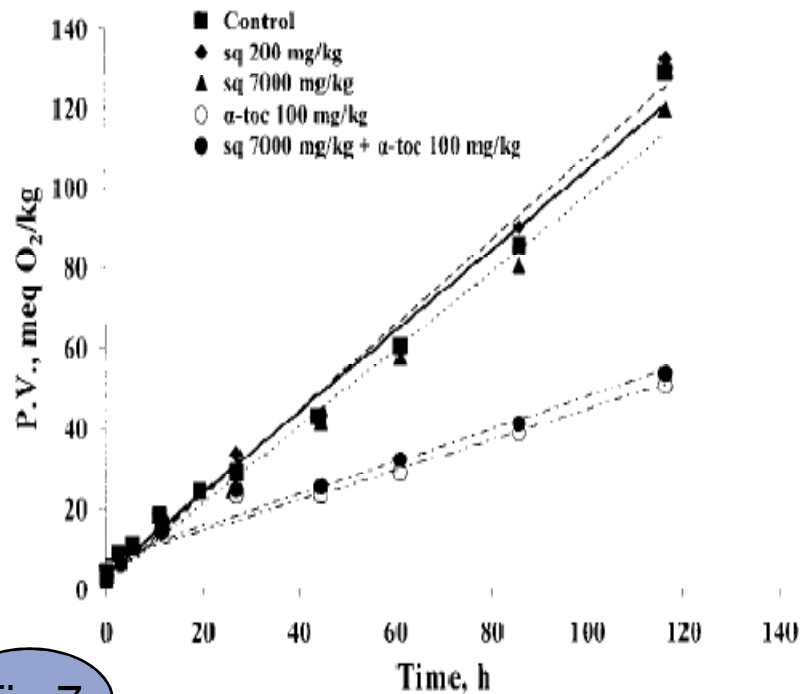


Fig.7

Effect of squalene,  $\alpha$ -tocopherol, and their combination on the photo-oxidation of olive oil stripped of antioxidants and pro-oxidants.

**Figure 7** shows that SQ did not have a protective effect on the lipid substrate and the activity of  $\alpha$ -tocopherol was not enhanced in its presence.

At 12100 lx (25 °C), a singlet oxygen quenching effect could not be attributed to SQ but its protective effect to  $\alpha$ -tocopherol was clearly seen.

- HPLC monitoring of the evolution of  $\alpha$ -tocopherol and SQ contents revealed that SQ participated in the oxidation process.

$\alpha$ -Tocopherol consumption during light exposure was lower in the presence of SQ (**Figure 8a**).

- much higher SQ consumption found for these samples (**Figure 8b**)
  - a new peak eluted earlier than SQ observed in the HPLC chromatograms that may be ascribed to an oxidation product.
- Findings possibly owed to the regeneration of  $\alpha$ -tocopherol radical by SQ as was also suggested by Kohno *et al.* (1995) and to the ability of SQ to trap two molecules of  $O_2$  forming stable cyclic hydroperoxides.

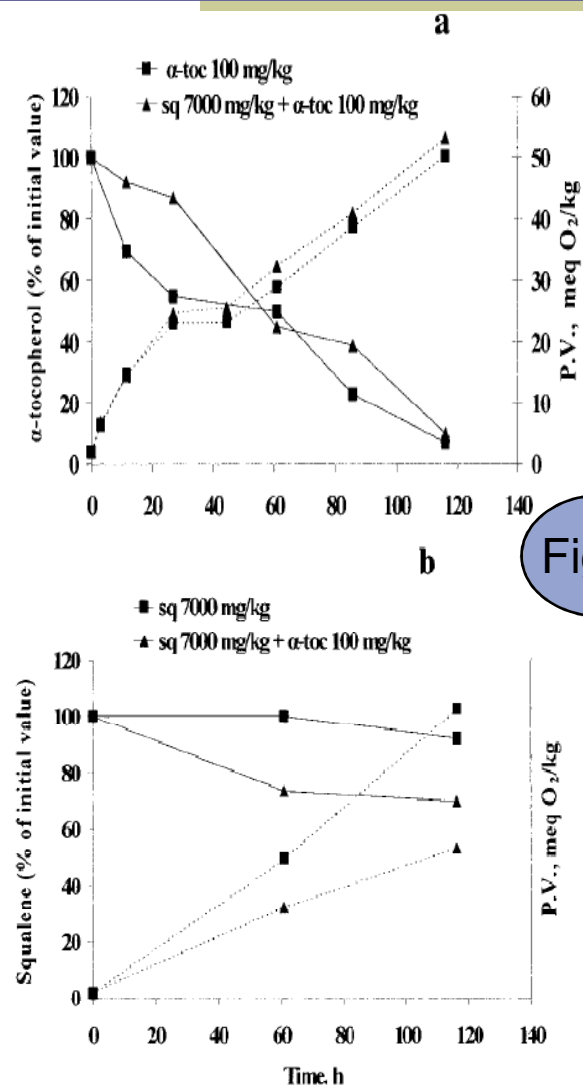



Fig. 8

## Rastrelli et al. (2002) observations were different from those made by Psomiadou & Tsimidou (2002b)

- During 12 months of storage the rate of the SQ, under the same storage conditions follows that of vitamin E, *from which it is protected*, at least in the first months of the study
  - Its content decreased significantly only after 6 months in half-empty bottles (Scallicelle VOO, -45.0%; Terzera VOO - 51.5%).
  - Diffused lighting does not appear to play a significant role in the SQ degradation (Scallicelle VOO, -19.3%, and Terzera VOO, -23.8%, after 12 months of storage in the filled colorless bottles at diffused lighting).
- *EXPERIMENTAL:*
  - *VOO samples were filtered and stored either in 20 colorless glass bottles (500 mL) or in 20 dark ones (500 mL).*
  - *Half the bottles were filled (3% headspace), while the remaining ones were half-empty (50% headspace).*
  - *The bottles were well taped and stored at room temperature and under diffused light to simulate the typical home storage conditions.*





SQ seems to act synergistically  
with different minor compounds of  
VOO during autoxidation and  
photoxidation

Data sometimes contradictory concerning  
its role

## Performance in other oils and fats?

- Hudson & Ghavami, 1984: slight activity in **lard** at 100 °C
- Psomiadou & Tsimidou, 1999: in lard as in OO; worse in sunflower oil
- Dessì, et al., 2002: At a molar ratio of 7:1 (PUFA:SQ) the inhibition of the oxidation process was 22% in the presence of linoleic acid and about 50% in presence of linolenic, arachidonic and docosahexaenoic acids tested against temperature-dependent autoxidation and UVA (ultraviolet A, 320–380 nm) mediated oxidation.

Findings suggest that the reaction of autoxidation is predominant and SQ acts mainly as peroxy radical scavenger.

**In this study SQ hydroperoxide did not act as prooxidant.**

The lack of prooxidant activity suggests that SQ peroxy radical is probably stabilized by resonance in its isoprenic structure.

## Squalene performance upon lipid heating ?

- Sims *et al.*, 1972
- Addition of **certain vegetable oil unsaponifiabiles** ( from olive, corn, wheat germ and *Vernonia anthelmintica* oils) to **safflower oil** protects it from oxidative polymerization during heating at frying temperature (180 °C, four 7h heating periods)
- The rate of destruction of polyunsaturates by oxidative polymerization was done by iodine value
- **The unsaponifiabiles were found to be effective.**
- The fraction responsible for this effect is largely sterol in nature.

As far as SQ is concerned (addition of 0.5 %)  
although it does make a significant contribution  
(retarded the degradation of unsaturated fatty acids  
-> limited the drop in iodine value),  
**it is probably not responsible for the entire effect.**

## Boskou & Morton, 1975

- **virgin olive oil samples** heated at 180 °C, 8 h a day with overnight cooling to room temperature.
- After 24, 48 and 72 h of heating the samples were removed and stored for analysis.

The amount of SQ  
was reduced from 550 mg/100 g of oil to 120 mg/100 g of oil after 72 h.

- In terms of percentage peak area, SQ made up 90 % of the total area of the hydrocarbon fraction in the fresh sample and **30 %** in the heated one.

## Malecka, 1991; 1994

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- The effect of SQ on the heat stability of **rapeseed oil**.
- The samples of rapeseed oil and both model lipids containing 0.4 % of SQ or without any additives (controls) were heated in open glass vessels at 170°C for 35 h.
- Concentration (0.10 to 1.0%) dependent activity was evidenced.
- After heating for 10h the amount of polar compounds increased to 13.3 % in the controls and to 10.4% in the samples containing SQ.

Albi *et al.*, 1997

- VOO, OO, sunflower oil, high oleic sunflower oil & lard
- 1. heating by microwave energy ( $170 \pm 10$  °C, 60 min), MH
- 2. heating in conventional electric oven ( $180 \pm 2$  °C, 120 min), CH
- 3. exposure to microwave energy (for 60 min, at intervals of 50s below 40 °C), M

Tab. 5

**Squalene Content (Parts per Million) in the Fats and Oils before and after Treatments<sup>a</sup>**

sample	total squalene	loss %	sample	total squalene	loss %
VOO	4700	0	SO	224	0
VOO-MH	3478	$26 \pm 0.5$	SO-MH	177	$21 \pm 0.4$
VOO-CH	4230	$9 \pm 0.2$	SO-CH	199	$11 \pm 0.2$
VOO-M	4700	0	SO-M	244	0
OO	3559	0	HOSO	175	0
OO-MH	2598	$27 \pm 0.5$	HOSO-MH	147	$16 \pm 0.3$
OO-CH	3203	$10 \pm 0.2$	HOSO-CH	163	$7 \pm 0.1$
OO-M	3559	0	HOSO-M	175	0

<sup>a</sup> Mean value of four determinations.

# Squalene performance upon lipid heating ?

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- Gertz et al., 2000
- Non-refined and refined vegetable fats and oils were heated at a temperature of 170 °C after adding water-conditioned silica gel for two hours.
- The data showed that the presence of natural substances such as SQ, sterol fraction, quercetin, oryzanol, and ferulic acid enhance the stability of vegetable oils at a higher temperature more than other synthetic compounds (BHT, BHA, ascorbic acid 6-palmitate and gallates).

## SQ upon oil heating and potato frying

*Abdalla et al., 1999*

- The addition of 1% of OO unsaponifiable matter to sunflower oil showed the highest effect in retarding the oxidation deterioration of oil during frying of potato chips.
- This protective effect was attributed to high levels of SQ,  $\Delta 7$ -avenasterol, and tocopherols.
- During ten frying days, the amount of squalene decreased to 79% and both tocopherols and  $\Delta 7$ -avenasterol to 69% in frying sunflower oil (tocopherol is not consumed at first, and only squalene disappears).
- Chips with high amounts of squalene, tocopherols, and sterols showed highest antioxidative stability during storage for 3 months at ambient temperature.



## SQ upon oil heating and potato frying

Kalogeropoulos et al., 2004; 2006

- sunflower oil, cottonseed oil, corn oil, soybean oil, palm kernel oil, palm oil, vegetable shortening oil - palm oil+sunflower oil+cottonseed oil-, and cooking fats sampled during deep-frying of potatoes from 21 restaurants in Athens, Greece, and in domestic frying oils [VOO, vegetable shortening & sunflower oil] used during the domestic pan frying and deep-frying of potatoes.
- **SQ appeared to be rather stable during frying.**
- Its retention in used restaurant seed oils and fats remained over 50% even after 30 h of frying while a high recovery of 84-96% was observed during the domestic deep-frying of potatoes in VOO.



A general agreement on  
contribution of SQ to oil stability  
at higher temperatures

TECHNOLOGICAL IMPORTANCE

# EPILOGUE

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- SQ is abundant in VOO to be neglected.
- Its importance in autoxidation and photoxidation of VOO needs further clarification.
- Its thermal stability is documented as well as its positive role upon oil heating.
- **STILL, A SYSTEMATIC WORK IS NEEDED TO ESTABLISH ITS NUTRIENT VALUE AND TECHNOLOGICAL IMPORTANCE.**

Thank you for attention

