

---

## **DIETARY EVALUATION OF VEGETABLES PAN-FRIED IN VIRGIN OLIVE OIL FOLLOWING THE GREEK TRADITIONAL CULINARY PRACTICE**

---

**NICK KALOGEROPOULOS  
DIMITRIS GRIGORAKIS  
ANASTASIA MYLONA  
ANGELIKI FALIREA  
NIKOLAOS K. ANDRIKOPOULOS**

Department of Science of Dietetics-Nutrition,  
Harokopio University, Athens, Greece

The aim of this work was the nutritional evaluation of fresh vegetables, pan-fried in virgin olive oil according to the traditional Greek culinary practices. The vegetables studied (potatoes, green peppers, zucchini, and eggplant) are traditionally consumed after pan-frying in virgin olive oil in Greece and other Mediterranean countries. The fried samples contained less moisture, more fat, and more energy when compared to the raw ones, while they were significantly enriched in squalene,  $\beta$ -sitosterol, campesterol, and stigmasterol. Their fatty acid composition reflected that of the olive oil they absorbed. The results obtained indicate that among other nutritional benefits of their consumption, vegetables fried in virgin olive oil appear to represent a healthy lipid profile in regard to fatty acid classes and their atherogenic and thrombogenic indices, while they furthermore provide an additional intake of monounsaturated fat, squalene, and phytosterols for Mediterranean peoples.

**KEYWORDS** Domestic pan-frying, virgin olive oil, vegetables, energy content, fatty acids, squalene, phytosterols

The authors would like to thank Mrs. Margarita Christea for her technical assistance.  
Address correspondence to Nick Kalogeropoulos, Harokopio University, 70 El. Venizelou Avenue, 176 71 Kallithea, Athens, Greece. E-mail: nickal@hua.gr

## INTRODUCTION

Vegetables, together with fruit and olive oil, hold a key position in Mediterranean diet (Simopoulos, 2001). The average consumption of vegetables in Greece ranges between 500 and 550 g per person per day (Trichopoulou et al., 2003).

Pan-frying of potatoes, green peppers, zucchini, and eggplant is a usual practice in Greece and other Mediterranean countries. The fresh vegetables are traditionally pan-fried in virgin olive oil (VOO) as they are, while zucchini and eggplant are in some cases blanketed with wheat flour or batter mix before frying. The fried vegetables are very popular in Greece and they accompany a wide variety of dishes like salads, greens, roasted meat, while in some cases they are used as ingredients for other Mediterranean recipes (eg imam, mousaka).

Frying is considered to have almost the same or even less effect on nutrient losses compared to other cooking methods (Bognár, 1998; Fillion & Henry, 1998), while the absorption of frying oils which are usually rich in vitamin E (Andrikopoulos et al, 1989) and unsaturated fatty acids (Fillion & Henry, 1998) usually increase the nutritive value of fried food. Among cooking oils, VOO is unique, being very rich in monounsaturated fatty acids (MUFA) and containing significant amounts of health-promoting microconstituents like tocopherols, squalene, polyphenols, and terpenoids (Boskou & Visioli, 2003; Owen et al., 2000).

Squalene is a triterpene primarily known as an intermediate in the biosynthesis of sterols in plants and animals (Kelly, 1999). It is widely distributed in nature and it is found in reasonable amounts in VOO ranging from 200–700 mg/100 g of oil (Smith, 2000). During the last decade, research has indicated that squalene may contribute to the reported anti-carcinogenic activity of olive oil (Kelly, 1999), especially for colon cancer in experimental animals (Smith, 2000) and humans (Rao et al., 1998) and together with phenolic compounds and oleic acid is probably responsible for the anti-inflammatory properties of olive oil (Kelly, 1999; Owen et al., 2000).

Phytosterols (or plant sterols) are natural dietary components, which can compete with dietary cholesterol to be absorbed by the intestines, resulting in lower serum cholesterol levels (Jones et al., 1997; Law, 2000). Phytosterols and especially  $\beta$ -sitosterol may also have some effect in cancer prevention, according to several reports that have documented their *in-vitro*, anti-tumor activity against benign prostatic hyperplasia (Wilt et al., 1999), prostate (von Holtz et al., 1998), breast (Awad et al.,

2000), and colon cancer (Awad et al., 1998). De Stefani et al. (2000) found a strong inverse relationship between the total intake of phytosterols and stomach cancer in humans. Virgin olive oil contains phytosterols at amounts varying between 113–265 mg/100 g oil. Campesterol, stigmasterol, and  $\beta$ -sitosterol are the main olive oil phytosterols, with  $\beta$ -sitosterol mounting up to 90–95% of total sterols (Gutierrez et al., 1999; Kiritsakis & Markakis, 1987).

Reports providing nutritional data for pan-fried vegetables are mostly focused on the interactions between fat and food during the deep-frying of potatoes and potato products as reviewed by Fillion and Henry (1998) and Dobarganes et al. (2000). To our knowledge there are no nutritional data for vegetables other than potatoes pan-fried in VOO. There is also a lack of data about the fate and distribution of VOO micro-constituents, like squalene and phytosterols, during the pan-frying of vegetables, including potatoes. This study was carried out to determine the proximate composition as well as energy, fatty acids, squalene, and phytosterols content of the vegetables that are usually consumed pan-fried by Greeks. Nutritional evaluation concerning the daily intake of squalene, phytosterols, fat, and fatty acid classes by consuming the fried vegetables and comparisons with nutrient intake data from Greece is also discussed. Finally, indices related to the atherogenic and thrombogenic potential of the pan-fried vegetables are presented.

## **MATERIALS AND METHODS**

### **Reagents and Chemicals**

Standards of  $\beta$ -sitosterol, stigmasterol, campesterol, squalene, 5- $\alpha$ -cholestane, and a mixture of 37 fatty acid methyl esters (FAME) were purchased from Sigma Chemicals Co. (St. Lewis, MO, USA). Butylated hydroxytoluene (BHT), boron trifluoride methanol solution (14%  $\text{BF}_3/\text{MeOH}$ ), and bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) were provided by Sigma (Steinheim, Germany). All the solvents used were of analytical grade and were obtained from Aldrich (Steinheim, Germany).

### **Sample Collection and Preparation**

The vegetables were purchased from the local market during spring 2005 and were immediately brought to the laboratory and washed. The potatoes

were peeled and cut in approximate uniform pieces (6 cm long and 0.9–1 cm thick). Green peppers were cut, cleaned, and sliced in pieces (8 cm long and 3–4 cm wide). Eggplants and zucchinis were sliced in round pieces with 6–7 cm diameter, and a thickness of 1 cm and 0.3 cm, respectively. The vegetables were subsequently wiped and pan-fried following the traditional frying practices. The frying was carried out in batch processes in fresh extra VOO that was purchased in sealed plastic bottles from the local market. Zucchini and eggplants were also fried blanketed with wheat flour, representing 5–8% w/w of the fresh vegetables, or with batter mix, representing 30% w/w of fresh vegetables. All frying probes were performed in the same stove using the same frying pan. The frying conditions are presented in Table 1. Temperature was not allowed to exceed 170°C and the vegetables were removed from the frying pan as soon as they were uniformly cooked without external burnings, and then they were placed in a clean dry grill for 5–10 min, allowing for the excess oil to drain. In order to estimate the amount of water loss and oil absorption, the weight of both oil and food before and after frying was recorded. After each frying operation, the used oil was replaced with fresh and the frying pan was thoroughly cleaned. Samples of raw and pan-fried oils and vegetables were taken for analysis. Samples of fresh and fried oils were kept under nitrogen in screw-capped vials at –20°C until analysis. Composite samples of vegetables were prepared by homogenizing 100–150 g of the respective raw and fried vegetables.

### **Moisture, Fat, and Energy Determination**

The homogenized vegetable samples were freeze-dried, sealed in plastic bags and kept at –20°C until further analysis. Moisture was calculated from the weight loss during freeze-drying. Fat was determined gravimetrically after extraction of lipids according to the method of Bligh and Dyer (1959). The extracted lipids were weighed, dissolved in hexane containing 20 µg/g BHT, transferred in screw-capped vials, and stored under nitrogen at –20°C.

The gross energy content was determined in the freeze-dried samples, by means of an IKA C4000 (IKA Analysentechnik, Heitersheim, Germany) adiabatic calorimeter.

### **Phytosterols Determination**

Phytosterols were determined by hot saponification of 100–200 mg of the freeze-fried samples with 0.5N NaOH in methanol, followed by methylation

Table 1. Variables Related to the Pan Frying of Vegetables in VOO

	Potato <sup>a</sup>	Green Pepper <sup>b</sup>	Zucchini <sup>c</sup>	Zucchini Floured	Zucchini Battered	Eggplant <sup>d</sup>	Eggplant Floured	Eggplant Battered
Oil quantity (L)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Surface of oil exposed to air (cm <sup>2</sup> )	706	706	706	706	706	706	706	706
Surface-to-volume ratio (cm <sup>-1</sup> )	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Amount of vegetable fried (g)	320	282	210	185	185	151	143	176
Amount of flour added (g)	—	—	—	15.3	—	—	13.6	—
Amount of batter added (g)	—	—	—	—	56.2	—	—	57.0
Food/oil ratio	1.3	1.1	0.8	0.8	1.0	0.6	0.6	0.9

VOO: virgin olive oil.

<sup>a</sup>*Solanum tuberosum* (in Greek: patata).

<sup>b</sup>*Capsicum annuum* (in Greek: piperia kerato).

<sup>c</sup>*Curcubita spp.* (in Greek: kolokithaki).

<sup>d</sup>*Solanum melongena* (in Greek: melitzana).

in  $\text{BF}_3/\text{MeOH}$ , as described by Hwang et al. (2003). The sterols containing unsaponifiable fraction was then extracted with 2 mL hexane, and 100  $\mu\text{L}$  5- $\alpha$ -cholestane 0.4% (w/v) was added as an internal standard. Aliquots of 0.1 mL of the above extracts were evaporated to dryness under nitrogen, derivatized to trimethylsilyl ethers by the addition of 250  $\mu\text{L}$  BSTFA at 70°C for 20 min (Klatt, 1995) and injected into the gas chromatograph.

An Agilent HP series GC 6890 (Avondale, PA, USA) equipped with flame ionization detector, split-splitless injector, and an HP 6890 autosampler were used for the determination of plant sterols. An aliquot (1  $\mu\text{L}$ ) of each silylated extract was injected into the gas chromatograph at a split ratio 1:20. Separation of the sterols was achieved on a SGE (Melbourne, Australia), BPX50 capillary column (30 m long, 0.25 mm internal diameter), coated with a 0.25- $\mu\text{m}$  thick film of 50% PH phenylmethylpolysiloxane. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. Injector and detector temperatures were set at 140°C and 290°C, respectively. The oven temperature was initially at 100°C, then increased at a rate of 20°C/min to a final temperature of 300°C where it remained for 15 min. The retention times of 5- $\alpha$ -cholestane, campesterol, stigmaterol, and  $\beta$ -sitosterol under these conditions were found to be 10.93, 11.61, 12.40, and 12.88 min, respectively. Quantification was carried out by constructing a reference curve after analyzing a series of standards containing different amounts of the sterols studied and a constant amount of internal standard (5  $\alpha$ -cholestane). The amount of internal standard and the volume of extracts were the same in all the samples and standards. All samples were analyzed in duplicate, exhibiting no more than 5% peak area variation.

### **Squalene Determination**

Squalene was determined in an aliquot of the Bligh-Dyer extract (containing 30–50 mg of fat), as well as in fresh VOO, after cold alkaline methylation, followed by GC/MS analysis, as described by Kalogeropoulos and Andrikopoulos (2004). Quantitation was carried out by means of external squalene standards (reference curve). All samples were analyzed in duplicate exhibiting less than 5% peak area variation.

### Fatty Acid Analysis

Fatty acids, in the form of their methyl esters, were determined by GC/MS in an aliquot of the Bligh-Dyer extract (containing 30–50 mg of fat), as described by Andrikopoulos et al. (2002).

### Indices

The atherogenic and thrombogenic potential of the oils and vegetables were evaluated by using the atherogenic index (AI) and the thrombogenic index (TI) introduced by Ulbricht and Southgate (1991), where  $AI = (12:0 + 14:0 + 16:0)/(n-3 \text{ PUFA} + n-6 \text{ PUFA} + \text{MUFA})$  and  $TI = (14:0 + 16:0 + 18:0)/(0.5 \text{ MUFA} + 0.5 n-6 \text{ PUFA} + 3 n-3 \text{ PUFA} + n-3 \text{ PUFA}/n-6 \text{ PUFA})$ . For TI and AI calculations, the fatty acid concentrations were expressed as g/kg of total fatty acids.

All chemical and chromatographic analyses were performed in duplicate. Data manipulation was performed by means of MS Excel.

## RESULTS AND DISCUSSION

### Changes in Moisture, Fat and Energy Content

In Table 2 the moisture, fat, and energy content of raw and fried vegetables, together with water loss and oil absorbed by the vegetables during pan frying is presented. Pan frying caused a significant water loss and fat increment due to the absorption of frying oil. As a result, both the fat and energy content of fried food increased significantly. Fat increased from 50 times in zucchini and green pepper up to 360 times in eggplant, while energy increased from 3 to 18 times.

Water loss ranged from 12.7% for zucchini to 52.2% for eggplants, while oil absorption ranged from 5.6–5.7% for green peppers and zucchini to 42.3% for eggplants. The high water loss and oil absorption of the eggplants could be attributed to the spongy texture of this vegetable. In regard to the frying technique, covering of vegetables with wheat flour or batter mix reduced the water loss and oil absorption in the case of eggplants, while the opposite was true in the case of zucchinis (Table 2). Texture differences between the two vegetables are probably the reason for this behavior.

Table 2. Indices on Raw and Pan Fried Vegetables in VOO

Vegetable	Moisture (g/100 g fw)	Fat (g/100 g fw)	Water Loss (A) (g/100 g fw)	Oil Absorbed (B) (g/100 g fw)	Energy (kcal/100 g fw)
Potato	83.6	0.1	—	—	65.4
Pan fried	50.3	10.9	39.5	14.5	240.5
Green pepper	94.6	0.1	—	—	23.3
Pan fried	86.6	5.2	28.9	5.6	74.1
Zucchini	94.7	0.1	—	—	20.1
Pan fried	83.5	5.1	12.7	5.7	85.0
Floured & pan fried	73.7	12.1	25.7	11.3	142.4
Battered & pan fried	56.5	16.1	40.3	15.7	271.2
Eggplant	92.8	0.1	—	—	25.2
Pan fried	43.6	35.9	52.2	42.3	446.6
Floured & pan fried	53.6	27.8	48.7	34.3	351.6
Battered & pan fried	58.3	16.5	38.6	23.9	193.1

VOO: virgin olive oil; fw: fresh weight;  $A = 100 \times [C - (D - E)]/C$ ;  $B = 100 \times E/D$ ; where C = vegetable before frying (g), D = vegetable after frying (g), E = oil absorbed (g) = oil before frying – oil after frying.



## Fatty Acids

The fatty acid content of raw and fried vegetables and the respective fresh and fried VOO are presented in Table 3. Polyunsaturated fatty acids (PUFA) were the main class in the lipids of raw vegetables, comprising 42.7–65.4% of total. Due to the very low lipid content of fresh vegetables, which was around 0.1% (Table 2), the VOO absorbed during frying resulted in deep change of their fatty acids profile towards that of VOO. As it is shown in Table 3, MUFA became predominant in all fried samples, representing more than 77% of total fatty acids.

**Table 3.** Fatty Acid Levels (g/100g of Total Fatty Acids) in Raw and Pan Fried Vegetables in VOO and in the Respective Fried VOO Used for the Pan Frying of Vegetables

		SFA	MUFA	<i>n</i> -6 PUFA	<i>n</i> -3 PUFA	<i>n</i> -6/ <i>n</i> -3
<b>Vegetables</b>						
Potato	Raw	31.4	19.2	40.1	6.5	6.2
	Pan fried	12.7	78.1	7.5	0.8	9.2
Green pepper	Raw	26.9	14.8	46.0	10.1	4.6
	Pan fried	13.6	76.8	8.3	1.0	8.4
Zucchini	Raw	27.2	5.4	14.8	50.7	0.3
	Pan fried	13.4	76.1	7.3	3.5	2.1
	Floured & pan fried	13.7	77.9	7.2	1.7	4.2
	Battered & pan fried	13.8	78.5	7.0	1.0	6.9
Eggplant	Raw	38.6	15.4	29.7	13.0	2.3
	Pan fried	12.9	79.6	6.7	0.6	11.7
	Floured & pan fried	13.7	79.6	6.9	0.6	11.5
	Battered & pan fried	13.7	78.6	6.9	0.6	11.6
<b>Oils</b>						
Fresh VOO		14.7	80.3	7.0	0.6	11.8
<b>Oils used for frying of:</b>						
Potato		13.1	78.8	6.6	0.5	12.5
Green pepper		13.4	78.5	6.8	0.6	12.1
Zucchini		13.4	78.6	6.7	0.5	12.4
Zucchini floured		14.1	78.1	6.7	0.5	12.8
Zucchini battered		13.5	78.9	6.6	0.5	12.7
Eggplant		13.5	78.6	6.6	0.6	12.1
Eggplant floured		13.8	78.9	6.6	0.5	12.3
Eggplant battered		13.5	78.0	6.4	0.5	13.7

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Monounsaturated fatty acids have received increasing attention during the last decades, as being potentially beneficial for the reduction of cardiovascular heart disease risk, as it was documented in the olive-oil consuming populations of the Mediterranean basin (Keys et al., 1986). As a result, the guidelines of the U.S. National Cholesterol Education Program (NCEP, 2001) have changed towards a higher dietary MUFA inclusion, most of which should be derived from vegetable sources, including plant oils and nuts. From this point of view, the MUFA predominance in the fried vegetables lipids may be beneficial.

The *n-6/n-3* ratios of fresh and pan-fried vegetables and VOO are shown also in Table 3. Frying and the subsequent absorption of VOO with *(n-6)/(n-3)* equal to 11.8, resulted in an increment of *(n-6)/(n-3)* ratios from 0.3–6.2 in raw vegetables to 2.1–11.7 in the respective fried ones (Table 3). With the exception of fried zucchinis, which showed *n-6/n-3* ratios from 2.1 to 6.9, close to the value of 5 indicated as the desirable ratio by Budowski and Crawford (1985), the respective values for the other pan-fried vegetables ranged from 8.4–12, being higher than 5 and almost reaching the value of 12, considered as typical for the Western diet (Simopoulos, 1999). Flour and batter resulted in higher *n-6/n-3* ratios in the case of zucchini, while in the case of eggplants, the *n-6/n-3* ratios were independent of the culinary practice.

The oils, fatty acid profiles were in all cases very similar to that of fresh VOO, and were not affected by the type of vegetable fried or the culinary practice applied (Table 3).

In order to express the intake of SFA, MUFA, and PUFA as a percentage of the energy provided by the pan-fried vegetables, the fatty acid data were converted to amounts (g) of individual fatty acids per 100 g fw, by applying the lipid conversion factors established by Weinrauch et al. (1977). The respective energy intakes were then calculated and are presented in Table 4, together with the intake of total fat and with the respective intake data for Greece (Moschandreas and Kafatos, 1999) and the guidelines of the U.S. National Cholesterol Education Program (NCEP, 2001). Fat provided 41–83% of total energy on an fw basis, the contribution being proportional to the amount of oil absorbed, exceeding in most cases the value of 42.7% reported for Crete, Greece (Moschandreas and Kafatos, 1999). The consumption of fried vegetables was found to supply less SFA, more MUFA and similar PUFA compared to the Greek national values (Table 4). The values were similar to the NCEP recommended values.

**Table 4.** Total Fat and Fatty Acid Composition Expressed as a Percentage of Energy in VOO Pan Fried Vegetables; Respective Nutrient Intake Data from Greece and the NCEP Guidelines Included for Comparison

Vegetable		Total Fat	SFA	MUFA	PUFA
Potato	pan fried	40.8	5.0	30.4	3.2
Green pepper	pan fried	63.2	8.3	46.6	5.6
Zucchini	pan fried	54.4	6.9	39.5	5.6
	floured & pan fried	76.2	10.0	56.7	6.5
	battered & pan fried	53.5	7.1	40.1	4.1
Eggplant	pan fried	83.1	10.3	62.8	5.7
	floured & pan fried	73.2	9.6	55.7	5.2
	battered & pan fried	78.1	10.2	58.5	5.6
Crete, Greece <sup>a</sup>		42.7	13.2	19.7	6.0
NCEP <sup>b</sup> guidelines		25–35	<7	<20	<10

VOO: virgin olive oil; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

<sup>a</sup>Moschandreas and Kafatos (1999).

<sup>b</sup>NCEP (2001).

## Squalene

The squalene content of raw and pan-fried vegetables, expressed as mg/100 g of fresh matter is given in Table 5. Raw vegetables contained traces of squalene (0.02–0.04 mg/100 g fw), while the squalene content of pan-fried samples ranged from 6.8–97.6 mg/100 g fw, a 2–3 orders of magnitude increase. The squalene content of fresh VOO used for frying was 616 mg/100 g. These findings together with a good linear relationship between the squalene content of the fried vegetables and the amount of oil absorbed are presented in Figure 1. It is obvious that the bulk of squalene present in fried samples originated from VOO as frying oil. The squalene content of fresh and fried oils, the percent recovery of squalene in the frying oils, and the overall squalene recoveries are presented in Table 6. The recoveries of squalene in frying oils were quite high, ranging from 55–95%, in agreement with the previously reported remarkable stability of squalene during domestic and commercial frying of potatoes (Kalogeropoulos and Andrikopoulos, 2004). The culinary practice affected the squalene retention, since the blanketing of vegetables with flour or batter resulted in better squalene recoveries (Table 6). The overall

**Table 5.** Squalene and Phytosterol Content of Raw and Pan Fried Vegetables in VOO (mg/100 g fw)

Vegetable		Squalene	Campesterol	Stigmasterol	$\beta$ -Sitosterol	Sum of Phytosterols Determined
Potato	Raw	0.04	0.1	0.1	1.2	1.4
	Pan fried	26.8	0.7	0.4	10.1	11.2
Green pepper	Raw	0.02	0.1	0.1	1.0	1.2
	Pan fried	15.6	0.8	0.4	4.8	6.0
Zucchini	Raw	0.03	0.1	0.1	0.7	0.9
	Pan fried	6.8	0.3	0.2	3.4	3.9
	Floured & pan fried	35.5	0.4	0.3	4.8	5.5
	battered & pan fried	42.2	1.1	1.1	15.7	17.9
Eggplant	Raw	0.04	0.2	0.1	0.9	1.2
	Pan fried	97.6	1.7	1.0	35.9	38.6
	Floured & pan fried	73.6	1.2	0.9	25.0	27.1
	Battered & pan fried	67.8	1.0	0.7	18.7	20.4

VOO: virgin olive oil; fw: fresh weight.

recoveries of squalene (taking into account both frying oil and food) followed a similar pattern (Table 6).

### Plant Sterols

The phytosterol content of raw and pan-fried vegetables, expressed as mg/100 g of fresh matter is given in Table 5. Raw vegetables contained small amounts of campesterol (0.1–0.2), stigmasterol (around 0.1), and  $\beta$ -sitosterol (0.7–1.2), the respective concentrations in fresh VOO being 3.1, 2.4, and 95.3. As a result of VOO absorption, the pan-fried samples were enriched in phytosterols, their concentrations increased by a factor of 2–11 for campesterol, 2–10 for stigmasterol, and 5–40 for  $\beta$ -sitosterol. The good linear relationships between the phytosterol contents and the oil absorbed by fried vegetables, shown in Figure 2, indicate that the phytosterols present in the pan-fried vegetables originate from the frying oil. The phytosterol content of the fresh and pan-fried VOO together with

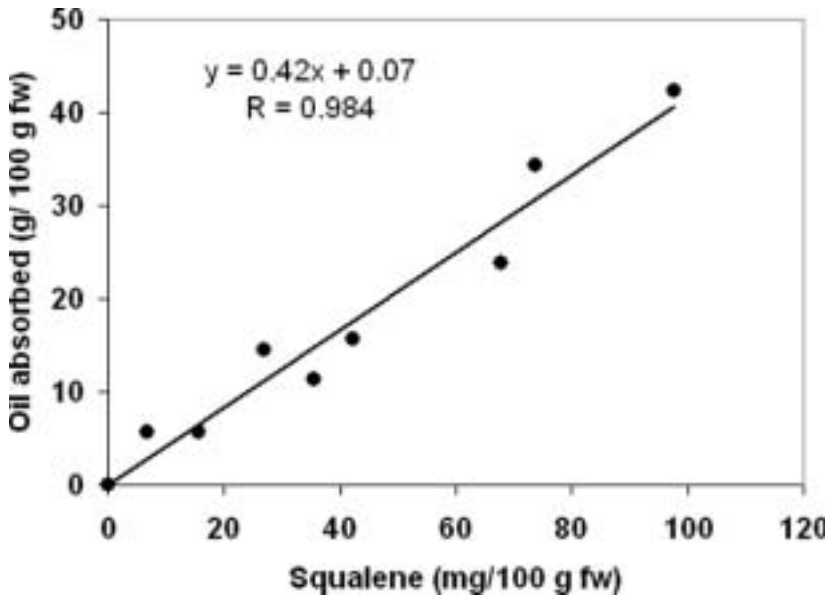


Figure 1. Relationship between squalene content and VOO absorbed in pan fried vegetables.

their recoveries in fried oil and their overall recoveries are given in Table 6. Good recoveries, ranging from 50% up to 87%, were observed for all the phytosterols determined.

### Dietary Intake of Squalene and Phytosterols by Consuming Pan Fried Vegetables

The dietary intake of squalene and phytosterols from consuming one serving (145 g) of vegetables pan-fried in VOO was calculated and is presented in Table 7. The squalene provided by one serving of vegetables fried in VOO ranged from 9.9 mg in fried zucchini to 141 mg in eggplants (average 70 mg per serving), covering the 3–47% (average 23%) of the estimated 200–400 mg daily squalene intake in the Mediterranean countries (Smith, 2000) and the 33–470% (average 230%) of the 30-mg daily squalene intake in the U.S. (Smith, 2000).

The phytosterol intake from the consumption of one serving (145 g) of vegetables pan-fried in VOO ranged from 5.8 mg per serving from fried zucchinis to 58.4 mg per serving from fried eggplants, representing the

**Table 6.** Squalene and Phytosterol Content of (1) Fresh VOO, (2) VOO Used for Frying the Vegetables Studied (mg/100 g Oil), (3) Their Retentions (%) in the Fried Oils, and (4) Their Overall Retentions<sup>a</sup> (%)

Oil Samples	Squalene			β-Sitosterol			Campesterol			Stigmasterol		
	Fried Oil Retention	Overall Retention	Retention	Fried Oil Retention	Overall Retention	Retention	Fried Oil Retention	Overall Retention	Retention	Fried Oil Retention	Overall Retention	Retention
Fresh VOO	615.6		98.5			3.1			2.4			
Fried VOO from:												
Potato	584.4	94.9	87.9	73.7	74.8	64.3	1.5	47.7	56.0	1.1	64.3	50.0
Green pepper	504.8	82.0	80.3	82.5	83.8	82.2	1.7	55.2	55.6	1.3	82.2	62.2
Zucchini	434.3	70.5	68.7	88.0	89.3	65.2	1.5	50.5	51.5	1.1	65.2	45.8
Zucchini floured	485.6	78.9	77.7	76.0	77.2	71.0	1.7	55.9	57.5	1.1	71.0	48.2
Zucchini battered	598.2	97.2	92.0	74.1	75.3	55.8	2.1	69.4	78.6	1.6	55.8	91.3
Eggplant	338.5	55.0	51.8	72.6	73.7	87.3	2.6	84.0	91.2	2.1	87.3	95.2
Eggplant floured	396.1	64.3	58.8	90.8	92.2	84.9	2.5	82.8	82.3	2.0	84.9	89.8
Eggplant battered	445.6	72.4	66.0	74.2	75.4	79.9	1.8	57.4	64.9	1.0	79.9	67.5

VOO: virgin olive oil; fw: fresh weight.

<sup>a</sup>calculated from squalene, β-sitosterol, campesterol, and stigmasterol present in both food and oil, before and after pan frying.

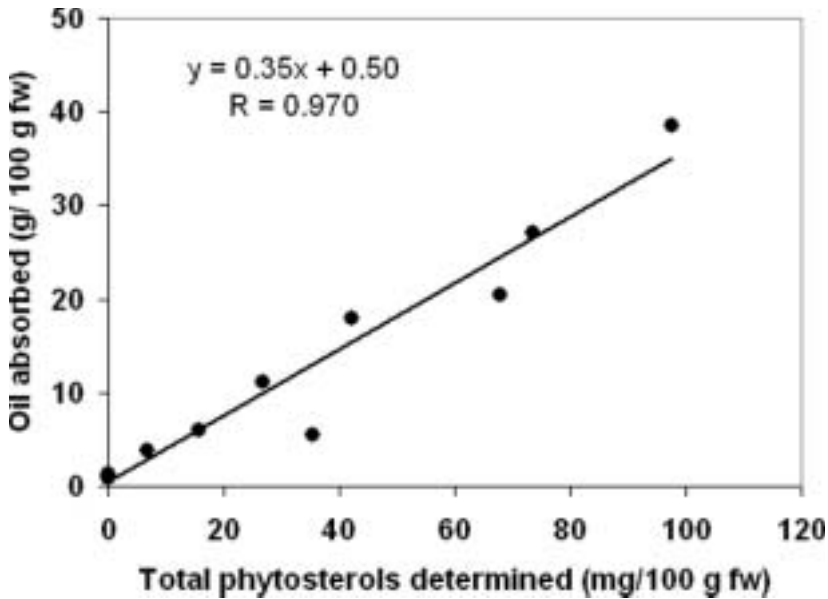


Figure 2. Relationship between the phytosterols content and VOO absorbed in pan fried vegetables.

2.3–23% of the daily plant sterols intake, which is around 250 mg (Law, 2000).

### Indices

The atherogenic and thrombogenic potential of the vegetables were evaluated by calculating the respective indices (Ulbricht and Southgate, 1991) and the results are presented in Table 8. The atherogenic index (AI) ranged between 0.25–0.30 for the raw and 0.11–0.13 for the pan-fried samples, which shows a 2-fold decrease. The thrombogenic index (TI) of the raw and pan-fried samples ranged between 0.24–0.44 and 0.22–0.25, respectively, showing a less-pronounced decrease. The AI of fresh and fried VOO were close to 0.13, while TI ranged from 0.22–0.24 (Table 8). The changes of AI and TI values in the fried samples are clearly the result of VOO absorption. In any case both AI and TI of the pan-fried samples were lower than 0.25.

**Table 7.** Dietary Intakes of Squalene, Campesterol, Stigmasterol, and  $\beta$ -Sitosterol Expressed as mg Per Serving<sup>a</sup> of the Pan Fried in VOO Vegetables

Vegetable		Squalene	Campesterol	Stigmasterol	$\beta$ -Sitosterol	Total Phytosterols Determined
Potato	pan fried	38.9	1.0	0.6	14.6	16.2
Green pepper	pan fried	22.5	1.2	0.6	7.0	8.7
Zucchini	pan fried	9.9	0.3	0.2	4.9	5.4
	floured & pan fried	51.4	0.6	0.4	7.0	8.0
	battered & pan fried	61.2	1.6	1.6	22.8	26.0
Eggplant	pan fried	141.4	2.5	1.5	52.1	56.0
	floured & pan fried	134.2	1.7	1.3	36.3	39.3
	battered & pan fried	98.0	1.5	1.0	27.1	29.6

VOO: virgin olive oil.

<sup>a</sup>One serving = 145g.**Table 8.** Atherogenic (AI) and Thrombogenic (TI) Indices (1) in Fresh and Pan Fried VOO and (2) in Raw and Pan Fried in VOO Vegetables

		VOO		Vegetables			
		AI	TI	AI		TI	
				Raw	Fried	Raw	Fried
Fresh VOO		0.13	0.24				
Fried VOO from:							
Potato	pan fried	0.13	0.22	0.28	0.11	0.44	0.22
Green pepper	pan fried	0.13	0.23	0.25	0.13	0.34	0.24
Zucchini	pan fried	0.13	0.22	0.29	0.13	0.24	0.23
	floured & pan fried	0.13	0.24		0.13		0.25
	battered & pan fried	0.13	0.23		0.13		0.25
Eggplant	pan fried	0.13	0.23	0.30	0.12	0.43	0.22
	floured & pan fried	0.13	0.23		0.13		0.23
	battered & pan fried	0.13	0.23		0.13		0.23

VOO: virgin olive oil.



## CONCLUSIONS

Pan-frying of vegetables in VOO resulted in significant water loss and an increase in oil and energy content, the extent of which depended on the type of vegetables fried and the culinary practice followed. The absorption of VOO caused a substantial change in the fatty acid profile of the fried vegetables, with monounsaturated fatty acids becoming predominant comprising more than 70% of total fatty acids.

Squalene and phytosterols, originating from the VOO, significantly enriched the pan-fried samples, their amounts being proportional to that of the oil absorbed. These VOO microconstituents were proven to be rather stable during pan frying. Their calculated recoveries ranged between 50–85%, and were to some extent affected by the culinary practice.

Squalene and phytosterols, provided by consuming a serving (145 g) of vegetables pan fried in VOO, were found to represent a significant fraction of the respective daily intakes, especially in the case of eggplants.

Furthermore, it was found that pan frying of vegetables in VOO decreased the thrombogenic and atherogenic potential of the food obtained.

From the data obtained in the present study, it is concluded that vegetables pan fried in virgin olive oil provide, among others, an additional intake of monounsaturated fat, squalene and phytosterols for Mediterranean peoples.

## REFERENCES

- Andrikopoulos, N. K., G. V. Z. Dedoussis, A. Falirea, N. Kalogeropoulos, and H. S. Hatzinikola (2002). Deterioration of natural antioxidant species of vegetable edible oils during the domestic deep-frying and pan-frying of potatoes. *International Journal of Food Science and Nutrition*, 53, 351–363.
- Andrikopoulos, N. K., M. N. Hassapidou, and A. G. Manoukas (1989). The tocopherol content of Greek olive oils. *Journal of the Science of Food and Agriculture*, 46, 503–509.
- Awad, A. B., A. C. Downie, and C. S. Fink (2000). Inhibition of growth and stimulation of apoptosis by beta-sitosterol treatment of MDA-MB-231 human breast cancer cells in culture. *International Journal of Molecular Biology*, 5, 541–545.
- Awad, A. B., R. L. von Holtz, J. P. Cone, C. S. Fink, and Y. C. Chen (1998). beta-Sitosterol inhibits growth of HT-29 human colon cancer cells by activating the sphingomyelin cycle. *Anticancer Research*, 18, 471–473.

- Bligh, A. C., and W. J. Dyer (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911–917.
- Bognár, A. (1998). Comparative study of frying to other cooking techniques influence on the nutritive value. *Grasas Aceites*, 49, 250–260.
- Boskou, D., and F. Visioli (2003). Biophenols in table olives. In M. P. Vaquero, T. Garcia-Arias, and A. Garbajal (Eds.) *Bioavailability of micronutrients and minor dietary compounds. Metabolic and technical aspects.* (pp. 161–169). Trivandrum, India: Research Signpost.
- Budowski, P., and M. A. Crawford (1985).  $\alpha$ -Linoleic acid as a regulator of the metabolism of arachidonic acid: dietary implications of the ratio *n-6:n-3* fatty acids. *Proceedings of the Nutrition Society*, 44, 221–229.
- De Stefani, E., P. Boffetta, A. L. Ronco, P. Brennan, H. Deneo-Pellegrini, J. C. Carzoglio, and M. Mendilaharsu (2000). Plant sterols and risk of stomach cancer: a case-control study in Uruguay. *Nutrition and Cancer*, 37, 140–144.
- Dobarganes, C., G. Márquez-Ruiz, and J. Velasco (2000). Interactions between fat and food during deep-frying. *European Journal of Lipid Science and Technology*, 102, 521–528.
- Fillion, L., and C. J. K. Henry (1998). Nutrient losses and gains during frying: a review. *International Journal of Food Science and Nutrition*, 49, 157–168.
- Gutiérrez, F., B. Jimenez, A. Ruiz, and M. A. Albi (1999). Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties picual and hojiblanca and on the different components involved. *Journal of Agricultural and Food Chemistry*, 47, 121–127.
- Hwang, B. S., J. T. Wang, and Y. M. Choong (2003). A simplified method for the quantification of total cholesterol in lipids using gas chromatography. *Journal of Food Composition and Analysis*, 16, 169–178.
- Jones, P. J., D. E. MacDougall, F. Ntanos, and C. A. Vanstone (1997). Dietary phytosterols as cholesterol-lowering agents in humans. *Canadian Journal of Physiology and Pharmacology*, 75, 217–227.
- Kalogeropoulos, N., and N. K. Andrikopoulos (2004). Squalene in fats and oils used for the domestic and commercial frying of potatoes. *International Journal of Food Science and Nutrition*, 55, 125–129.
- Kelly, G. S. (1999). Squalene and its potential clinical use. *Alternative Medicine Reviews*, 4, 29–36.
- Keys, A., A. Menotti, M. J. Karvonen, C. Aravanis, H. Blackburn, R. Buzina, B. S. Djordjevic, A. S. Dontas, F. Fidanza, M. H. Keys, D. Kromhout, S. Nedeljkovic, S. Punsar, F. Seccareccia, and H. Toshima (1986). The diet and 15-year death rate in the Seven Countries Study. *American Journal of Epidemiology*, 124, 903–915.
- Kiritsakis, A., and P. Markakis (1987). Olive oil: a review. *Advances in Food Research*, 31, 453–482.

- Klatt, L. V. (1995). Cholesterol analysis in foods by direct saponification-gas chromatographic method: Collaborative study. *Journal of AOAC International*, 78, 75–79.
- Law, M. R. (2000). Plant sterol and stanol margarines and health. *British Medical Journal*, 320, 861–864.
- Moschandreas, J., and A. Kafatos (1999). Food and nutrient intakes of Greek (Cretan) adults. Recent data for food-based dietary guidelines in Greece. *British Journal of Nutrition*, 81, S71–S76.
- National Cholesterol Education Program (NCEP) (2001). Expert panel on detection, evaluation and treatment of high blood cholesterol in adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (adult treatment panel III). *Journal of the American Medical Association*, 285, 2486–2497.
- Owen, R. W., A. Giacosa, R. Haubner, G. Wurtele, B. Spiegelhalter, and H. Bartsch (2000). Olive-oil consumption and health: the possible role of antioxidants. *Lancet Oncology*, 1, 107–112.
- Rao, C. V., H. L. Newmark, and B. S. Reddy (1998). Chemopreventive effect of squalene on colon cancer. *Carcinogenesis*, 19, 287–290.
- Simopoulos, A. P. (1999). Genetic variation and evolutionary aspects of diet. In A. Papas (Ed.) *Antioxidants in nutrition and health*. Boca Raton: CRC Press, pp. 65–88.
- Simopoulos, A. P. (2001). The Mediterranean diets: what is so special about the diet of Greece? The scientific evidence. *Journal of Nutrition*, 131, 3065S–3073S.
- Smith, T.J. (2000). Squalene: potential chemopreventive agent. *Expert Opinion on Investigational Drugs*, 9, 1841–1848.
- Trichopoulou, A., T. Costacou, C. Bamia, and D. Trichopoulos (2003). Adherence to a Mediterranean diet and survival in a Greek population. *New England Journal of Medicine*, 348, 2599–2608.
- Ulbricht, T. L. V., and D. A. T. Southgate (1991). Coronary heart disease: seven dietary factors. *Lancet*, 338, 985–992.
- von Holtz, R. L., C. S. Fink, and A.B. Awad (1998). beta-Sitosterol activates the sphingomyelin cycle and induces apoptosis in LNCaP human prostate cancer cells. *Nutrition and Cancer*, 32, 8–12.
- Weihrauch, J. L., L. P. Posati, B. A. Anderson, and J. Exler (1977). Lipid conversion factors for calculating fatty acid contents of foods. *Journal of the American Oil Chemists Society*, 54, 36–40.
- Wilt, T. J., R. MacDonald, and A. Ishani (1999). beta-Sitosterol for the treatment of benign prostatic hyperplasia: a systematic review. *British Journal of Urology International*, 83, 976–983.

