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Recovery and distribution of natural antioxidants (α-tocopherol, polyphenols and terpenic acids) after pan-frying of Mediterranean finfish in virgin olive oil

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Abstract

Samples of eight finfish representing the most popular fish species in Greece were pan-fried in virgin olive oil according to the Greek traditional culinary practice. Analyses for polyphenols, hydroxy pentacyclic triterpene acids (HPTA) and α-tocopherol were performed in the fresh and fried oils and fish. Polyphenols and HPTA were determined by GC/MS and α-tocopherol by HPLC. Nine polyphenols were determined in the frying oil samples; six of them were also found in fried fish. The terpenic acids oleanolic, maslinic and ursolic were also determined in frying oils and fried fish. No polyphenols and no HPTA were detectable in raw fish, while α-tocopherol was present in all samples. Besides water loss and oil absorption, pan frying caused the partial loss of all the antioxidants studied in the fried oils, as well as their enrichment in the fried fish. The overall retention of α-tocopherol in the fried oil and fish ranged from 30% to 80%, the respective values for polpyphenols and HPTA ranging between 51–87% and 46–88%. The polarity of the antioxidants studied, seems to affect to some extent their partition between the frying oil and the water-containing fish.

Keywords: Polyphenols; Terpenic acids; α-Tocopherol; Mediterranean finfish; Pan-frying; Virgin olive oil

1. Introduction

Fish constitute an important part of the Mediterranean diet (Simopoulos, 2001), the average consumption of fish in Greece being between 22 and 26 g per day per person (Trichopoulou, Costacou, Bamia, & Trichopoulos, 2003).

Fried foods are consumed world-wide with sustainable popularity as a result of their unique and delicious sensory characteristics (Gertz, 2000). The frying process is considered to have almost the same or even less effect on nutrient losses than other cooking methods (Bognár, 1998; Fillion & Henry, 1998). Furthermore, the frying oils absorbed by fried foods normally increase the nutritive value of food, since they are rich in vitamin E (Andrikopoulos, Hassapidou, & Manoukas, 1989) and unsaturated fatty acids (Fillion & Henry, 1998). Among cooking oils and fats, virgin olive oil (VOO) is unique both because it is very rich in monounsaturated fatty acids and because it contains significant amounts of health-promoting micronutrients like squalene, polyphenols, terpenoids and tocopherols (Boskou & Visioli, 2003; Owen et al., 2000).

Pan-frying of fish in VOO is the usual practice in Greece and other Mediterranean countries. Mediterranean fish and mollusks pan-fried in VOO were found to represent food of high nutritive value with a healthy fatty acids profile and enriched in VOO originating squalene (Kalogeropoulos, Andrikopoulos, & Hassapidou, 2004).

During the last decades, accumulating literature data have indicated a key role for free radicals as major contributors to

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aging and to degenerative diseases such as cancer, cardiovascular disease, cataracts, immune system decline and brain dysfunction (Ames, Shigenaga, & Hagen, 1990; Percival, 1998; Young & Woodside, 2001). Antioxidants are compounds capable of stabilizing, or deactivating free radicals before they attack cells and biological targets; therefore, their availability is crucial for maintaining optimal cellular and systemic health and well-being (Percival, 1998).

Tocopherols are considered as the most important lipid phase natural antioxidants, which prevent lipid peroxidation by scavenging radicals in membranes and lipoprotein particles (Esterbauer, Dieber-Rotheneder, Striegl, & Waeg, 1991). Olive oils produced in Greece contain α-tocopherol, the vitamin E homologue with the highest biological activity (Chow Ching, 1985), in quantities varying from 1.2 to 43 mg/100 g (Kiritsakis & Markakis, 1987; Psomiadou, Tsimidou, & Boskou, 2000).

Polyphenols are the most abundant natural antioxidants in our diet (Boskou & Visioli, 2003). Many studies have indicated their antioxidant capacity with respect to oxidative alterations due to free radicals and other reactive species (Soler-Rivas, Espin, & Wichers, 2000) and the oxidation of low-density lipoproteins (Andrikopoulos, Kaliora, Assimopoulou, & Papageorgiou, 2002). Polyphenol intake has been associated with lower risk of coronary heart disease (Keys, 1995; Simopoulos, 2001; Tapiero, Tew, Nguyen, & Mathe, 2002; Trichopoulou & Lagiou, 1997), some types of cancer (Simopoulos, 2001; Trichopoulou & Lagiou, 1997), inflammation (Tapiero et al., 2002; Trichopoulou & Lagiou, 1997) and inhibition of platelet-activating factor activity (Andrikopoulos, Antonopoulou, & Kaliora, 2002).

VOO furthermore contains significant amounts of hydroxy pentacyclic terpenic acids (HPTA), particularly oleanolic, maslinic and ursolic acids (Pérez-Camino & Cert, 1999). During the last two decades, pharmacological studies of oleanolic and ursolic acids indicated that they have many beneficial effects, notably hepato-protection, antiinflammation, antitumor-promotion and antihyperlipidemia (Liu, 1995), while maslinic and ursolic acids exhibit anti-HIV activity (Xu, Zeng, Wan, & Sim, 1996). Recently, the triterpenic fraction extracted from *Pistacia lentiscus* var. *Chia* (Chios mastich gum) was proved to exert an antioxidant/antiatherogenic effect (Dedoussis et al., 2004).

There are several reports concerning the loss of tocopherols (Andrikopoulos, Dedoussis, Falirea, Kalogeropoulos, & Hatzinikola, 2002; Andrikopoulos, Kalogeropoulos, Falirea, & Barbagianni, 2002; Barrera-Arelano, Ruiz-Mendez, Velasco, Márquez-Ruiz, & Dobarganes, 2002; Gordon & Kourimská, 1995) or polyphenols during frying (Andrikopoulos et al., 2002; Andrikopoulos et al., 2002; Gómez-Alonso, Fregapane, Salvador, & Gordon, 2003) or frying simulating operations (Brenes, Garcia, Dobarganes, Velasco, & Romero, 2002). To our knowledge there are no data concerning the distribution of these health promoting micronutrients in pan-fried fish, which are actually consumed by humans. There are also no data about the HPTA behavior during pan-frying. This study was carried out to determine the recovery and distribution of natural antioxidants during pan-frying of Mediterranean finfish in VOO. A nutritional evaluation concerning the intake of polyphenols, tocopherols and HPTA by consuming the pan-fried fish is also discussed.

2. Materials and methods

2.1. Reagents and chemicals

Methanol, hexane, acetone, acetonitrile, chloroform, ethyl acetate of analytical grade, methanol HPLC grade, bis-(trimethylsilyl)-trifluoroacetamide (BSTFA), quercetin, 3-(4-hydroxyphenyl)-1-propanol, homovannilic alcohol and cinnamic acid were obtained from Aldrich (Steinheim, Germany). Tyrosol, protocatechuic acid and caffeic acid, were obtained from Fluka (Steinheim, Germany). 4-Hydroxy-benzoic acid, *p*-hydroxy-phenylacetic acid, ursolic acid, vanillin, *p*-coumaric acid, and ferulic acid, were obtained from Sigma (Steinheim, Germany). Vanillic acid was obtained from Serva (Heidelberg, Germany). Hydroxytyrosol by organic synthesis was kindly donated by the Laboratory of Food Chemistry and Technology, Aristotle University of Thessaloniki.

2.2. Sample collection and preparation

The finfish chosen for the present study, shown in Table 1, are common in the Aegean and Mediterranean Seas. They are very popular in Greece and are consumed exclusively (*Atherina boyeri*, *Boops boops*, *Spicara smaris*, *Trachurus trachurus*) or almost exclusively (*Engraulis encrasicholus*, *Mullus barbatus*, *Merluccius merluccius*, *Sardina pilchardus*) pan-fried in VOO. The fish were obtained from the local fish market during the spring of 2005. The quantity of each sample was 2 kg. The fish were immediately brought to the laboratory, their lengths and weights were recorded, they were washed with cold water, scales were removed and they were then prepared according to the traditional Greek culinary practice. The washed and prepared fish were blanketed with wheat flour and they were pan fried in fresh extra VOO, which was purchased in sealed plastic bottles from the local market. Frying was performed in a frying pan (30 cm diameter, 5 cm depth), which contained 300 ml VOO. The food/oil ratio ranged from 0.5 to 0.9 and the temperature was not allowed to exceed 170 °C to achieve uniform cooking without external burning. The fish were turned and cooked on both sides and were removed from the frying pan as soon as they were browned. Then they were placed in a clean dry grill for 5–10 min, allowing for the excess oil to drain. In order to estimate water loss and oil absorption, the weights of both oil and food prior to 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 fish were taken for analysis. Oil

Table 1
Biometric data and pretreatment of the Mediterranean finfish studied

Species systematic name	English common name	Greek common name	Length[a] (cm)	Weight[a] (g)	Pretreatment[b]
Atherina boyeri	Sand smelt	Atherina	5.5 ± 0.3	1.2 ± 0.3	1
Spicara smaris	Picarel	Marida	10.4 ± 0.5	11.0 ± 1.7	1,2,3,4
Engraulis encrasicholus	Anchovy	Gavros	12.4 ± 0.8	13.5 ± 1.9	1,2,3,4
Mullus barbatus	Striped mullet	Koutsomoura	15.0 ± 0.5	40.9 ± 3.6	1,2,3,5
Boops boops	Bogue	Gopa	16.5 ± 0.9	44.9 ± 6.8	1,2,3
Trachurus trachurus	Scad	Savridi	17.9 ± 0.8	52.1 ± 7.9	1,2,3,4,5
Merluccius merluccius	Hake	Bakaliaraki	19.5 ± 0.9	49.9 ± 9.6	1,2,3,4,5
Sardina pilchardus	Sardine	Sardela	19.9 ± 0.9	63.9 ± 4.3	1,2,4

[a] Means ± SD.
[b] 1, Wash and drain; 2, scales removal; 3, gut removal; 4, head removal; 5, bone removal (1–3 prior to pan-frying; 4,5 prior to composite sample preparation). All fish were floured before frying.

samples were kept under nitrogen in screw-capped vials at −20 °C, until analysis. Composite samples of raw and pan-fried fish were prepared by homogenizing 50–100 g of the edible portion from each species. The composite samples were freeze-dried, sealed in plastic bags and kept at −20 °C until further analysis.

2.3. Tocopherol extraction and HPLC analysis

α-Tocopherol was isolated from the food samples by extracting 0.5 g sample five times with 5 ml hexane containing 20 mg/kg butylated hydroxytoluene. The extracts were combined, hexane was evaporated to dryness under vacuum and the residue was dissolved in a mixture of chloroform:isopropanol (3/1, v/v). Aliquots of 20 μl of these solutions were subjected to reversed phase high performance liquid chromatography (RP-HPLC) analysis by an HPLC system (Agilent Technologies, model HP 1050, Waldbronn, Germany) combined with auto-sampler, diode array detector HP-1050, fluorescence detector HP 1046A and data software. A quaternary solvent system was used consisting of water acidified with *o*-phosphoric acid at pH 3, methanol, acetonitrile and propanol-2, with gradient elution on a Nucleosil C18 100-5 (125 mm × 4.6 mm) (MZ, Wöhlerstr., Mainz, Germany) at a flow rate of 1 ml/min as follows: initially 90% methanol/10% water (pH 3) to 95% methanol/5% water (pH 3) in 10 min; 100% methanol in 10 min; isocratical for 5 min; 20% acetonitrile/20% methanol/60% propanol-2 in 1 min; isocratic for 10 min; and finally to initial conditions in 4 min. A 10 min post run for the system equilibration was used. Quantification was carried out by a reference curve constructed by analyzing standard α-tocopherol solutions.

2.4. Polyphenols and terpenic acids extraction

The phenolic and terpenic compounds were extracted from oil and food samples with methanol, which has been reported to be superior to the mixtures of methanol/water (Owen et al., 2000). Oil (1 g) or dry food samples (0.5 g) were extracted three times with 5 ml methanol, the extracts were combined, methanol was evaporated under vacuum and the residue was dissolved in 2 ml acetonitrile, followed by washing twice with 3 ml hexane. The acetonitrile was evaporated under vacuum and the residue was dissolved in 1 ml methanol. Aliquots of 0.1 ml of the above extracts were evaporated to dryness under nitrogen, derivatized by addition of 250 μl BSTFA at 70 °C for 20 min (Soleas, Diamandis, Karumanchiri, & Goldberg, 1997) and injected into a gas chromatograph.

2.5. Gas chromatography/mass spectrometry (GC/MS) of polyphenols and terpenic acids

An Agilent (Wallborn, Germany) HP series GC 6890N coupled with a HP 5973 MS detector (EI, 70 eV), split–splitless injector and an HP 7683 autosampler were used for the determination of polyphenols and terpenic acids. An aliquot (1 μl) of each silylated extract was injected into the gas chromatograph at a split ratio 1:20. Separation of sample was achieved using an HP-5 MS capillary column (5% phenyl–95% methyl siloxane, 30 m × 0.25 mm × 250 μm). Helium was used as a carrier gas at a flow rate of 0.6 ml/min. The injector and transfer line temperature were set at 280 and 300 °C, respectively. The oven temperature program was: initial temperature 70 °C for 5 min, 70–130 °C at 15 °C/min, 130–160 °C at 4 °C/min, held for 15 min and finally 170–300 °C at 10 °C/min where it was held for 15 min. A selective ion monitoring (SIM) GC/MS method was applied for detection of 28 target polyphenolic compounds and terpenic acids. Detection of polyphenols was based on the ±0.05 RT presence of target and qualifier ions of the standard polyphenols at the predetermined ratios. Target and qualifier ions (T, Q1 and Q2) for the 25 polyphenolic compounds were set as following: vanillin: 194, 209; cinnamic acid: 205, 220; tyrosol: 179, 267, 282; homovanilic alcohol: 209, 179; *p*-hydroxy-benzoic-acid: 267, 223, 193; *p*-hydroxy-phenyl-acetic acid: 252, 296, 281; *p*-hydroxy-phenyl-propanoic acid: 192, 310; vannilic acid: 297, 267, 312; hydroxytyrosol: 267, 370; homovanillic acid: 326, 267, 311; protocatechuic acid: 193, 355, 370; 3,4-dihydroxy-phenyl-acetic acid: 384, 267, 179; *p*-coumaric acid: 308, 293, 219; *o*-coumaric acid: 293, 308, 147; gallic acid: 281, 458, 443; ferulic acid: 338, 323, 308; syringic acid: 327, 342, 312; caffeic acid: 396, 219, 381; sinapic acid: 368, 353, 338; resveratrol: 444,

445, 443; epicatechin: 368, 355, 474; catechin: 368, 355, 474; kampherol: 559, 560; chlorogenic acid: 345, 307, 324; quercetin: 647, 559, 575. Three terpenic acids which have been detected in olive oils (Pérez-Camino & Cert, 1999) were also quantified as following: oleanolic acid: 203, 320, 482; ursolic acid: 203, 320; maslinic acid: 203, 320.

Identification of chromatographic peaks was made by comparing the retention times and ratios of two or three fragment ions of each polyphenolic compound with those of reference compounds, while quantitation was carried out by using 3-(4-hydroxyphenyl)-1-propanol as internal standard at target ion *m/z* 206 and qualifiers 191 and 179. Internal standard quantitation was performed based on a series of 9 standard mixtures of the polyphenolic and terpenic compounds containing the same quantity of internal standard as that of samples. Two control standards (low and high) were analyzed every 10 samples and if found to be outside of 10% of calibration standards, recalibration was performed. Quantitation of maslinic acid was based on oleanolic acid response factors.

Linearity was obtained for all 25 target phenolic compounds in the range of quantitation limit and up to 20-fold concentration for each polyphenolic compound. Very good linearity was also obtained in the range of quantitation limit and up to 1000-fold concentration for each terpenic acid.

3. Results and discussion

3.1. Water loss and oil absorption

In Table 2, the water loss and oil absorbed by the finfish studied after pan-frying are presented. Water loss ranged from 38.6% fresh weight (fw) for *S. pilchardus* to 66.7% fw for *A. boyeri*, while oil absorption ranged from 13.5% fw for *B. boops*, to 43.6% fw for *A. boyeri*. It is noteworthy that the higher water loss and oil absorption were observed for fish with the smaller length and weight like *A. boyeri* and *E. encrasicholus* (Tables 1 and 2). Both fish weight and length are known to affect water loss and oil absorption since they affect the total contact area between oil and food. This is in agreement with previously reported frying experiments with six of the eight fish species studied (Kalogeropoulos et al., 2004).

Table 2
Water loss and oil absorbed (g/100 g fw) in the edible portion of finfish pan-fried in VOO

Species	Water loss (A)	VOO absorbed (B)
A. boyeri	66.7	43.6
E. encrasicholus	58.7	31.1
B. boops	39.2	13.5
M. barbatus	51.7	22.0
S. smaris	51.4	25.7
T. trachurus	52.2	25.6
M. merluccius	48.7	23.1
S. pilchardus	38.6	17.6

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

3.2. α-Tocopherol

As shown in Table 3, all the oil and food samples before and after frying contained α-tocopherol. The α-tocopherol content of the VOO used for the frying operations was 17.2 mg/100 g, a value that is within the range of 12–25 mg/100 g reported by Psomiadou et al. (2000) as typical for Greek commercial VOO. The α-tocopherol content of raw fish was less than 0.1 mg/100 g fw. The VOO absorbed by fish during frying resulted in a significant enrichment of α-tocopherol in the fried samples, ranging from 1.93 mg/100 g fw in *A. boyeri* to 2.97 mg/100 g fw in *M. barbatus* representing an increment of 1–2 orders of magnitude (Table 3). The α-tocopherol recoveries in frying oils, presented in Table 4, ranged from 28.8% to 80.8% (average 59.2%), being almost identical to the respective overall α-tocopherol recovery (calculated from the tocopherol content of both food and oil) which ranged from 28.4% to 80.5% (average 59.2%). In an attempt to compare the α-tocopherol remaining in the frying oil to that absorbed by the fried food, the α-tocopherol content of fried fish was expressed as mg/100 g of absorbed oil and it was compared with the respective frying oil content. The results, as given in Table 4, indicate that in regard to α-tocopherol, the composition of the oil remaining in the frying pan is similar to that of the oil absorbed by the food. Therefore,

Table 3
α-Tocopherol content of (1) fresh VOO (mg/100 g oil), (2) raw finfish and (3) pan-fried in VOO finfish (mg/100 g fw)

Samples	α-Tocopherol
Fresh VOO	17.2
A. boyeri	
Raw	0.06
Pan-fried	1.93
E. encrasicholus	
Raw	0.08
Pan-fried	2.78
S. smaris	
Raw	0.03
Pan-fried	2.79
M. barbatus	
Raw	n.d.
Pan-fried	2.97
B. boops	
Raw	0.10
Pan-fried	2.92
T. trachurus	
Raw	0.03
Pan-fried	2.29
M. merluccius	
Raw	0.01
Pan-fried	2.36
S. pilchardus	
Raw	0.03
Pan-fried	2.61

VOO, virgin olive oil; fw, fresh weight; n.d., not detected.

Table 4
Recovery (%) and content (mg/100 g oil) of α-tocopherol in (1) fried VOO and (2) absorbed[b] VOO by the fried fish (mg/100 g oil)

	α-Tocopherol recovery in fried VOO	Overall[a] α-tocopherol recovery	α-Tocopherol content in fried VOO	α-Tocopherol content in absorbed[b] VOO
A. boyeri	28.8	28.4	5.0	4.2
E. encrasicholus	39.0	43.8	6.7	12.1
S. smaris	53.5	53.3	9.2	8.8
M. barbatus	80.8	80.5	13.9	13.4
B. boops	76.2	74.0	13.1	10.6
T. trachurus	51.2	51.1	8.8	8.7
M. merluccius	77.9	74.8	13.4	10.1
S. pilchardus	65.9	67.7	11.3	14.5

[a] Calculated from α-tocopherol present in both food and oil, before and after pan-frying.
[b] Calculated from the weights of oil and food before and after frying and corrected by substracting the α-tocopherol present in fresh food: VOO, virgin olive oil.

it is possible to estimate the α-tocopherol content of fried fish by simply analyzing the frying oil.

3.3. Polyphenols

Among 25 phenolic compounds checked, the polyphenols detected in most of the frying oil samples, were the nine presented in Table 5. Tyrosol was the major phenolic species in fresh VOO, comprising 71% of total polyphenols determined, followed by hydroxytyrosol which comprised 17.7%. Pan-frying caused a significant loss of polyphenols in VOO, ranging from 48.1% for *M. merluccius* to 63.5% for *A. boyeri*. As shown in Table 6, raw fish did not contain any detectable amounts of polyphenols while a significant enrichment of these compounds in fried fish was observed. Vanillin, ferulic acid and *p*-coumaric acid were not detected in these samples as compared to Table 5. The polyphenol content of frying oils ranged from 0.97 mg/100 g oil in *M. merluccius* to 1.38 mg/100 g oil in *A. boyeri* (Table 5). The phenolic content of pan-fried fish ranged from 0.72 mg/100 g fw in *M. merluccius* to 2.14 mg/100 g fw in *A. boyeri* (Table 6) following a linear relationship with the amount of oil absorbed. Tyrosol was the predominant phenolic compound, both in frying oils and in the edible portion of pan-fried fish, followed by hydroxytyrosol (Tables 5 and 6).

The retention of nine polyphenols in the frying oils ranged from 36.5% to 51.9% (average 46.0%). The overall retention of the nine polyphenols, calculated from the amounts present in the oil and the food before and after frying, ranged from 51.3% to 86.6% (average 72.9%), being in all cases higher than the respective retention in the frying oils. In order to compare the polyphenols retention in the fried fish and in the frying oils, the polyphenols concentrations in the fried fish were expressed as mg/100 g of absorbed oil and were compared with their respective concentrations in the frying oils. The results for total nine polyphenols and for the predominant tyrosol, are given in Table 7. As it can be calculated, the concentrations of polyphenols in the oils absorbed by fried fish were 3.5–8 (average 5.7) times higher than the respective concentrations in the frying oil. The deterioration of antioxidants during frying is something expected since they react rapidly with lipid radicals and are thereby consumed. The observed distribution indicates that either polyphenols survive better from the frying process when they are absorbed by the fish

Table 5
Polyphenols in VOO (mg/100 g oil) used for frying the finfish studied and their percent retention

No.	Phenolic compounds	Fresh VOO	Fried VOO after frying of							
			A. boyeri	*E. encrasicholus*	*B. boops*	*M. barbatus*	*S. smaris*	*T. trachurus*	*M. merluccius*	*S. pilchardus*
1	Tyrosol	1.90	1.02	1.06	0.77	0.83	0.94	0.97	0.74	0.85
2	Hydroxytyrosol	0.47	0.20	0.10	0.20	0.23	0.07	0.19	0.09	0.29
3	Homovanillic alcohol	0.11	0.07	0.09	0.08	0.08	0.08	0.09	0.08	0.08
4	*p*-Hydroxy-benzoic acid	0.02	0.01	0.02	Tr.	0.01	0.01	Tr.	0.01	Tr.
5	*p*-Hydroxy-phenylacetic acid	0.02	0.01	Tr.	Tr.	0.01	Tr.	Tr.	Tr.	0.01
6	Vanillin	0.04	0.03	0.01	0.01	0.02	0.02	0.02	0.02	0.02
7	Vannilic acid	0.03	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01
8	Ferulic acid	0.03	0.01	Tr.	Tr.	Tr.	Tr.	Tr.	0.01	Tr.
9	*p*-Coumaric acid	0.02	0.01	Tr.	Tr.	0.01	Tr.	0.01	0.01	0.01
	Total of the nine polyphenols	2.66	1.38	1.32	1.10	1.21	1.14	1.30	0.97	1.28
	Retention (%) of the nine polyphenols		51.9	49.6	41.4	45.5	42.9	48.9	36.5	48.1

VOO, virgin olive oil; Tr., traces (<0.005 mg/100 g).

Table 6
Polyphenols in the edible portion of finfish (mg/100 g fw) before and after pan-frying in VOO

No.	Phenolic compounds	Raw fish	Pan-fried fish							
			A. boyeri	*E. encrasicholus*	*B. boops*	*M. barbatus*	*S. smaris*	*T. trachurus*	*M. merluccius*	*S. pilchardus*
1	Tyrosol	n.d.	1.92	0.90	1.83	1.68	1.63	1.57	0.63	1.06
2	Hydroxytyrosol	n.d.	0.10	0.03	0.13	0.13	0.15	0.08	0.03	0.07
3	Homovanillic alcohol	n.d.	0.08	0.05	0.08	0.07	0.06	0.07	0.04	0.07
4	*p*-Hydroxy-benzoic acid	n.d.	0.01	0.01	0.01	0.01	0.01	0.01	Tr.	0.01
5	*p*-Hydroxy-phenylacetic acid	n.d.	0.01	0.01	0.01	0.01	0.01	0.01	Tr.	0.01
6	Vanillin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7	Vannilic acid	n.d.	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01
8	Ferulic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9	*p*-Coumaric acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total of the nine polyphenols	–	2.14	1.00	2.06	1.91	1.87	1.74	0.72	1.22
	Overall retention[a] (%) of the nine polyphenos	–	67.6	65.4	67.4	86.2	86.6	85.7	51.3	72.9

VOO, virgin olive oil; Tr., traces (<0.005 mg/100 g); n.d., not detected.
[a] Calculated from polyphenols present in both food and oil, before and after pan-frying.

Table 7
Distribution of (1) total of the nine polyphenols of Tables 5 and 6 and tyrosol in the fried VOO (mg/100 g oil) and (2) in the VOO absorbed[a] by the fried fish (mg/100 g oil)

	Fried VOO		Absorbed VOO	
	Total of the nine polyphenols	Tyrosol	Total of the nine polyphenols	Tyrosol
A. boyeri	1.38	1.02	5.38	4.40
E. encrasicholus	1.32	1.06	7.85	6.64
S. smaris	1.10	0.77	7.45	5.88
M. barbatus	1.21	0.83	9.89	7.63
B.. boops	1.14	0.94	8.58	6.40
T. trachurus	1.30	0.97	5.60	4.40
M. merluccius	0.97	0.74	3.40	2.72
S. pilchardus	1.34	0.85	7.73	5.99

[a] Calculated by the weights of oil and food before and after frying: VOO, virgin olive oil.

or that a diffusion of the relatively polar polyphenols towards the water rich fish tissue takes place. Sacchi et al. (2002) reporting on the partition of VOO phenolic compounds in oil–brine mixtures during thermal processing for fish canning, observed an absolute increment of tyrosol and hydroxytyrosol in the water–oil system after thermal treatment and suggested that this was the result of hydrolysis of tyrosol and hydroxytyrosol esters, which consequently migrated towards the water phase, a finding that was confirmed by Brenes et al. (2002) who studied the influence of the thermal processing of oil/water mixtures on the VOO polyphenols. Whichever the mechanism is, the result is that VOO originating phenolic compounds are present in the fried fish in amounts higher than those expected by the quantity of frying oil absorbed. Such a finding indicates that in order to estimate the polyphenolic content of pan-fried fish it is not enough to analyze only the frying oil, but the fried fish itself should be considered.

3.4. Terpenic acids

As shown in Table 8, the fresh VOO used for frying contained significant amounts of the terpenic acids oleanolic, maslinic, and ursolic, their concentrations being comparable to those reported in the literature (Pérez-Camino & Cert, 1999). Raw fish did not contain any detectable amounts of HPTA (Table 8). Pan-frying resulted in partial loss of HPTA from oils and their enrichment in fried fish (Table 8). The sum of the three HPTA concentrations in the edible portion of pan-fried fish ranged from 2.9 mg/100 g fw in *M. merluccius* to 12.6 mg/100 g fw in *A. boyeri* being, proportional to the amount of oil absorbed. The overall retention of the three HPTA, calculated from the amounts present in oil and food before and after frying, ranged from 45.5% to 88.1% (average 70.9%). The recovery of these HPTA in fried VOO was in all cases higher, ranging from 46.7% to 91.2% (average 75.9%). Comparison of HPTA concentrations in the fried oils and in the oils absorbed by the food, given in Table 9, revealed that their concentrations in the frying oils were 1.4–9.4 (average 3.7) times higher than their respective concentrations in the oil absorbed by the fried fish. The above distribution is opposite to that observed in the case of polyphenols (Section 3.3). Without excluding the possibility of a greater HPTA loss in the fried fish tissue, the observed distribution could be attributed to the more lipophilic nature of HPTA, which prefer to remain in the oil phase rather than migrating towards the water-rich fish tissue. Furthermore, when HPTA were examined separately it was observed that their relative abundances in the fried oils and in fried fish were different as presented in Table 8. Maslinic acid concentrations in fried fish were lower than those expected from its abundance in frying oils. Maslinic acid appears to be more lipophilic than the other two HPTA, a behavior that can be attributed to its molecular structure. Maslinic acid is the

Table 8
HPTA (1) in the fresh and pan-fried VOO (mg/100 g oil), (2) in the edible portion of pan-fried finfish (mg/100 g fw), (3) their recovery (%) in the frying VOO and (4) their overall[a] recovery (%)

	Oleanolic acid	Maslinic acid	Ursolic acid	Total of the three HPTA	Recovery of the three HPTA in fried VOO	Overall recovery of the three HPTA in fried VOO
Fresh VOO	36.8	27.6	1.32	65.7		
Fried VOO for						
A. boyeri	31.1	24.2	0.94	56.2	85.5	81.1
E. encrasicholus	15.8	14.5	0.39	30.7	46.7	45.5
S. smaris	20.1	24.0	0.19	44.3	67.4	62.7
M. barbatus	33.4	17.9	0.91	52.2	79.5	75.6
B. boops	32.8	20.7	0.84	54.4	82.7	75.2
T. trachurus	24.6	20.8	0.78	46.1	70.2	65.5
M. merluccius	31.1	23.8	0.57	55.5	84.4	73.8
S. pilchardus	33.9	25.3	0.75	59.9	91.2	88.1
Raw finfish	n.d.	n.d.	n.d.	–	–	–
Fried finfish						
A. boyeri	12.3	0.09	0.18	12.6		
E. encrasicholus	3.3	0.06	0.06	3.4		
S. smaris	4.9	0.04	0.06	5.0		
M. barbatus	7.0	0.04	0.10	7.1		
B. boops	6.0	0.03	0.06	6.1		
T. trachurus	6.4	0.07	0.12	6.6		
M. merluccius	2.8	0.08	0.04	2.9		
S. pilchardus	6.7	0.03	0.07	6.8		

HPTA, hydroxy pentacyclic triterpene acids; VOO, virgin olive oil.
[a] Calculated from HTPA present in both food and oil, before and after pan-frying.

only acid among the HTPA studied containing two adjacent hydroxyl groups that can participate in endomolecular hydrogen bond formation, thus reducing its hydrophilic potency as compared to the other two HPTA. The above findings indicate that, as in the case of polyphenols, it is not correct to estimate the HPTA content of fried fish, by analyzing the fried oil, but the fried fish should also be analyzed.

3.5. Distribution of antioxidants between fried oil and food

The comparison of α-tocopherol, polyphenols and terpenic acids concentrations in the fried oils and in the oils absorbed by the food is given in Tables 4, 7 and 9, respectively, and as already discussed in the previous sessions, provide indications that the distribution of the antioxidants studied between the frying oil and the water-containing food is – to some extent – controlled by differences in their polarity: the more polar polyphenols enriched the fried fish, the less polar HPTA remained mainly in the oil phase, while the intermediate polarity α-tocopherol was distributed more uniformly between oil and fried fish. These findings are summarized in Table 10.

Table 9
Distribution of the three HPTA of Table 8 (1) in the fried VOO and (2) in the VOO absorbed[a] by the pan-fried fish (mg/100 g oil)

	Fried VOO	Absorbed VOO
A. boyeri	56.2	41.0
E. encrasicholus	30.7	16.5
S. smaris	44.3	15.1
M. barbatus	52.2	18.8
B. boops	54.4	13.0
T. trachurus	46.1	10.1
M. merluccius	55.5	5.9
S. pilchardus	59.9	24.3

HPTA, hydroxy pentacyclic triterpene acids; VOO, virgin olive oil.
[a] Calculated from the weights of oil and food before and after frying.

3.6. Dietary intake of antioxidants by consuming pan fried fish

The dietary intake of antioxidants determined by consuming one serving (130 g) of fish pan-fried in VOO is presented in Table 11. The α-tocopherol provided by one serving (130 g) of pan-fried fish ranged from 2.5 mg in *A. boyeri* to 3.9 mg in *M. barbatus*, covering the 17–26%

Table 10
Ratios of the concentrations of antioxidants in fried VOO (mg/100 g oil)/antioxidants in the VOO absorbed by fried fish (mg/100 g oil)

	α-Tocopherol	Total of the nine phenols (Table 5)	Total of the three HPTA (Table 8)
A. boyeri	0.8	3.9	0.7
E. encrasicholus	2.4	6.0	0.5
S. smaris	1.0	6.8	0.3
M. barbatus	1.0	8.1	0.4
B. boops	0.6	7.5	0.2
T. trachurus	1.0	4.3	0.2
M. merluccius	0.8	3.5	0.1
S. pilchardus	1.3	5.8	0.4
Mean	1.1	5.7	0.4
SD	0.6	1.7	0.2

HPTA, hydroxy pentacyclic triterpene acids; VOO, virgin olive oil.

Table 11
Dietary intake of the antioxidants determined in fish pan-fried in VOO expressed as mg per serving[a]

	α-Tocopherol	Total of the nine polyphenols	Total of the three HPTA	Total antioxidants
A. boyeri	2.5	2.8	16.4	21.7
E. encrasicholus	3.6	1.3	4.4	9.3
S. smaris	3.6	2.7	6.5	12.8
M. barbatus	3.9	2.5	9.2	15.6
B. boops	3.8	2.4	7.9	14.2
T. trachurus	3.0	2.3	8.6	13.8
M. merluccius	3.1	0.9	3.8	7.8
S. pilchardus	3.4	1.6	8.8	13.8

[a] One serving = 130 g fried fish.

(average 22%) of the recommended daily dietary intake of vitamin E which has been set to 15 mg for healthy adults (Mahann & Escott-Stump, 2004). The intake of phenolic compounds by the consumption of one serving (130 g) of fish pan-fried in VOO ranged from 0.9 mg per serving in *M. merluccius* to 2.8 mg per serving in *A. boyeri*. The intake of HPTA was higher ranging from 3.8 mg per serving in *M. merluccius* to 16.4 mg per serving in *A. boyeri*. The phenolics together with HTPA provided by consuming one serving of pan-fried fish could cover 23–77% (average 41%) of the daily antioxidant intake as compared to the 23–28 mg of flavones and flavanones intake reported for The Netherlands and Denmark (Ross & Kasum, 2002) and around 4–16% (average 9%) if compared to the value of 118.6 mg reported as the daily average flavonoid intake from a traditional Greek plant-based diet (Vasilopoulou, Georga, Joergensen, Naska, & Trichopoulou, 2005). The sums of α-tocopherol, polyphenols and HPTA discussed here, ranged from 7.8 to 21.7 mg per serving (Table 11), covering a significant fraction of the daily antioxidants intake.

4. Conclusions

From the results obtained the following conclusions can be drawn:

- Pan frying of Mediterranean finfish in VOO resulted in partial loss of the antioxidants studied in the fried VOO, while their concentrations in the fried fish samples increased when compared with the respective raw ones.
- A significant overall retention in both fried VOO and fried fish of α-tocopherol (30–80%), polyphenols (52–87%) and HPTA (46–88%) was observed.
- Most of α-tocopherol and practically all the polyphenols and HPTA present in fried fish originate exclusively from VOO, confirming its value as a frying oil.
- The amount of antioxidants provided by consuming a serving (130 g) of fish pan-fried in VOO represents a significant portion of the respective daily intakes.
- Differences in the antioxidants polarity seem to govern – in some extent – their distribution between the frying oil and the water-containing food.
- In order to gain information for the fate and distribution of health promoting micronutrients originating from the frying oils, one should analyze both oils and food before and after frying.

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