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Chemical Composition of Greek Avgotaracho Prepared from Mullet (*Mugil cephalus*): Nutritional and Health Benefits

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Crude composition, lipid composition, and tocopherols, ascorbic acid, cholesterol, phytosterols, and squalene content together with fatty acids and antiplatelet activities of total, neutral, and polar lipids of avgotaracho (wax-covered, dried, and salted *Mugil cephalus* roe) were studied and compared with those of similar products. Wax and steryl esters accounted for 63.7% of roe lipids followed by phosphatidylcholine (PC), which comprised 20.3%. Wax esters were rich in saturated fatty alcohols, monounsaturated fatty acids, and long chain ω3 highly unsaturated fatty acids (HUFA). The fatty acid distribution in roe total and neutral lipids was similar to that of wax esters, while in polar lipids, the ω3 HUFA predominated. Avgotaracho provides significant amounts of protein, fat, α-tocopherol, ascorbic acid, and PC, certain amounts of squalene and phytosterols, and cholesterol at levels comparable to hens' eggs. Total, polar, and neutral lipids of avgotaracho exhibited a strong inhibition of platelet activating factors and thrombin, with polar lipids being more active. The results obtained indicate that avgotaracho is a food of high nutritive value, rich in protein and lipids with a healthy lipid profile in terms of ω3/ω6 ratio and major fatty acid classes, while the antiplatelet activity of its oil indicates a putative antithrombotic potential.

KEYWORDS: ***Mugil cephalus***; **roe**; **lipid composition**; **nutrition**; **benefits**; **platelet aggregation**

INTRODUCTION

Fish eggs are commonly referred to as roe, particularly when they are included in the original ovarian sac, to distinguish them from caviar—the best-known form of fish roe products—which is the salt-cured and preserved eggs of aquatic animals that have been separated from the supporting connective tissue. A large number of highly valued salted, smoked, boiled, or canned products are made from fish roe, and currently roe is involved in expanding international and domestic markets (*1*).

Striped mullet (*Mugil cephalus*) roe traditionally was used for the production of delicacies such as karasumi in Japan, botargo in Italy (Sardinia), and avgotaracho in Greece (*2, 3*). Japanese karasumi is consumed either as salted and cured whole ovaries or skeins or as a salted cured single egg product (*1*). In Italy and Greece, cured mullet roe is consumed sliced, as an appetizer, while in Italy it is additionally consumed grated, to season pasta (*2*). Besides Greece, Italy, and Japan, processed mullet roes are popular in France, Taiwan, Turkey, and several African countries; however, to our knowledge, no data concerning the typical consumption of such products exist. Dried mullet roe is yellowish red and has a chewy mouth feel due to the large quantity of wax esters, which comprised up to 70% of lipids (*3, 4*). Mullet roe is considered a highly nutritive food, having well-balanced proteins with essential amino acids and containing significant amounts of ω3 HUFA, such as 20:5ω3 (EPA) and 22:6ω3 (DHA) (*4*), known to play an important role in the prevention of cardiovascular diseases (*5*).

In Greece and Italy, salted and dried mullet roes are regional specialties, for which documented history goes back at least to the 17th century, and manufacturing procedures of drying ovarian sacs of fish seems to be connected to ancient techniques introduced by the Phoenicians into the Mediterranean area (*2*). During the last few decades, Greek avgotaracho has been gaining a wider reputation as a gourmet product. It is prepared from carefully collected intact ovarian sacs, weighing preferably ∼250−350 g, representing ∼17% of the fish weight. Mullet spawning season in the Mediterranean is from July to October, while the best season for mullet roe collection in Greece is considered to be the period after mid-August. The ovarian sacs are washed and covered with coarse sea salt for 2−3 h. They are subsequently put into casts to obtain their final shape and are air-dried under controlled conditions for 3−4 days. During this period, the roes lose ∼25% of their initial water content, while keeping their color and tenderness. For better preservation, the final product is covered with several (usually eight) layers of melted beeswax. The coating of avgotaracho with beeswax

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differentiates it from other similar products, as it reduces further water loss and minimizes contact with air.

Although a certain amount of data is available for the composition of raw *M. cephalus* roe (*6–8*), scarce data are available for the macro- and microconstituent composition of salted mullet roe (*4*) and commercial food preparations (*2, 3*), and there are no data at all concerning Greek avgotaracho. Moreover, no data concerning the bioactivity of fish roe lipids toward platelet aggregation and especially against the platelet-activating factor (PAF) have been published yet, although the lipid fraction of fish roes contains several constituents such as tocopherols, polyunsaturated fatty acids (*1, 4*), and gangliosides (*9*) with a known antiplatelet activity (*10–13*). The PAF, identified as 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine (*14*), is a strong thrombotic and inflammatory mediator (*15*) that has been reported to play a major role in atherosclerosis development (*16, 17*). PAF action could be controlled by PAF-acetylhydrolase or by PAF-antagonists either endogenously or derived from foods.

To our knowledge, this is the first report on the crude composition, minor constituents such as tocopherols, ascorbic acid, cholesterol, phytosterols, and squalene, as well as lipid composition and fatty acid composition of selected lipid classes, including wax esters, of mullet roe prepared as Greek avgotaracho. Furthermore, this is the first report on the antiplatelet activity of fish roe lipids.

MATERIALS AND METHODS

Reagents and Chemicals. Ascorbic acid, 2,4-dinitrophenylhydrazine, trichloroacetic acid (TCA), butylated hydroxytoluene (BHT), boron trifluoride in methanol (14% BF_3/MeOH), squalene, cholesterol, 5-α-cholestane, β-sitosterol, campesterol, stigmasterol, methyl heneicosanoate, methyl eicosapentaenoate, methyl docosahexaenoate, synthetic PAF (β-acetyl-γ-*O*-alkyl(16:0)-L-α-phosphatidylcholine), thrombin from bovine plasma, and bovine serum albumin-free fatty acid were purchased from Sigma Chemicals. A standard mixture of 37 fatty acid methyl esters (FAME) was purchased from Supelco. α-Tocopherol and δ-tocopherol were obtained from Aldrich (Steinheim, Germany), and γ-tocopherol was purchased from Fluka (Steinheim, Germany). Hexane, chloroform, propanol-2, and acetonitrile of HPLC grade as well as silica gel G were provided from Merck (Darmstadt, Germany). Bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) was obtained from Aldrich. All other solvents used were of analytical grade.

Sample Preparation. Three waxed, salted, and dried avgotaracho mullet roes—each one consisting of two skeins—obtained from different wild fish caught in the Ionian Sea (western Greece) were delivered frozen to the laboratory. According to the manufacturer, immediately after removal from the fish, the roes were kept in ice, and their processing started in less than 24 h. The processed roes were ∼18 cm long, having a cross-sectional diameter of 2−5 cm. The average weight of the waxed final product was 316.6 ± 24.0 g, with beeswax representing ∼15% w/w. As soon as the samples arrived in the laboratory, they were sealed under nitrogen in plastic bags and stored at −80 °C. Prior analysis samples were thawed and—after removing the wax cover—they were homogenized by gentle mixing by hand.

Crude Composition and Energy Determination. A portion of roe homogenate served for total lipids determination, while another portion was freeze-dried for 48 h, homogenized by a mixer, sealed in plastic bags, and kept at −40 °C until further analysis. Freeze-dried samples were used for the determination of ash, protein, and energy contents. Freeze-drying served also for moisture determination, as the water content of freeze-dried samples was found to be <1%. The ash content of freeze-dried samples was determined by programmed temperature incineration in a muffle furnace.

Total protein was calculated in the freeze-dried samples from Kjeldahl nitrogen using a 5.70 conversion factor (*18*). Kjeldahl nitrogen was determined by employing a Buchi (Zurich, Switzerland) B426 digesting apparatus and B316 steam-distillation apparatus. The gross energy content was determined in the freeze-dried samples, by means of an IKA C4000 (IKA Analysentechnik, Heitersheim, Germany) adiabatic calorimeter.

Fat content was determined gravimetrically in 20 g portions of fresh homogenate after extraction and purification of total lipids (TL) according to the method of Folch et al. (*19*). The extracted lipids were weighed, diluted in hexane, and separated into two portions to one of which BHT was added to a final concentration of 20 mg/L. The hexane solutions were stored in screw-capped vials under nitrogen at −40 °C.

Lipid Analysis. Mullet roe lipids were separated into polar (PL) and neutral (NL) fractions by counter current distributions in a binary system formed by mixing three volumes of pre-equilibrated petroleum ether and one volume of pre-equilibrated 87% ethanol (*20*). After solvent evaporation by applying reduced pressure, the two fractions were weighed, and the ethanolic fraction containing the PL was dissolved in chloroform/methanol (1:1 v/v), while the petroleum ether fraction containing NL was dissolved in petroleum ether. Both lipid fractions were separated into two portions to one of which BHT was added to a final concentration of 20 mg/L. The lipid solutions were stored in screw-capped vials under nitrogen at −40 °C.

The composition of NL and PL was determined by thin layer chromatography (TLC) alongside known standards using 20 cm × 20 cm glass plates coated with a 0.5 mm thick layer of silica gel G. Plates with PL were developed with chloroform/methanol/H_2O (65:35:6 v/v/v), while plates with NL were developed with petroleum ether/diethyl ether/glacial acetic acid (80:20:1 v/v/v). Separated lipid fractions were detected by spraying the developed plates with 3% cupric acetate in 8% phosphoric acid, followed by charring at 160 °C for 20 min (*21*), and were quantified by measuring the optical density of the corresponding spots. For this purpose, the charred plates were photographed with a digital camera, and the images were analyzed by Gel-Pro Analyzer V 3.1 for Windows (Media Cybernetics, Inc. Silver Spring, MD).

As wax esters comprise the major class of mugil roe lipids, wax and the coeluting steryl esters were isolated from the roe NL by preparative TLC and were recovered from the plates by scraping the corresponding bands—made visible by iodine vapors—and extracting them according to the Bligh−Dyer method (*22*). After solvent removal, wax and steryl esters were dissolved in hexane containing BHT and kept at −40 °C.

Fatty Acids and Fatty Alcohols Analysis. Fatty acids of TL, NL, and PL were determined by GC/MS, after their derivatization to methyl esters. Methyl esters of the fat component fatty acids (FAME) were prepared in screw-capped vials containing 10−20 mg of lipid samples after hot saponification with 2 mL of 0.5 M KOH in methanol, for 15 min at 90 °C, followed by methylation of fatty acids with 1.5 mL of BF_3/MeOH for 2 min at 90 °C (*23*). A saturated sodium chloride solution (5 mL) was then added, and FAMEs were extracted by hexane (2 mL). After vortexing and centrifuging at 800*g* for 10 min, aliquots (1 mL) of the hexane extracts together with 0.1 mL of internal standard solution (methyl heneicosanoate, 1 mg/mL) were transferred to GC vials and injected into the GC instrument. FAMEs were separated on a 50 m × 0.22 mm inner diameter BPX 70 capillary column, coated with a 0.25 μm film of cyanopropyl silicone provided by SGE (Melbourne, Australia), using an Agilent Technologies HP 6890 (Avondale, PA) gas chromatograph equipped with an autosampler and with a MSD-5972 mass selective detector, as previously reported (*24*).

HUFA—namely 20:5ω3 (EPA), 22:5ω3 (DPA), and 22:6ω3 (DHA)—were additionally quantitatively determined in total lipids extract by constructing reference curves with standard methyl esters of EPA and DHA and using methyl heneicosanoate as internal standard. DPA was quantified by means of a DHA reference curve.

The fatty acids and fatty alcohols of wax and steryl esters also were determined by GC/MS, after hot saponification followed by methylation of fatty acids with BF_3/MeOH—essentially as described in the previous paragraph—and silylation of fatty alcohols. For preparing the trimethylsilyl ether (TMS) derivatives of fatty alcohols, appropriate amounts of the hexane extracts were transferred to autosampler vials and evaporated to dryness under nitrogen, 0.25 mL of BSTFA containing 1% v/v trimethylchlorosilane was added, and the vials were sealed and

heated for 20 min at 70 °C. The fatty acids (as FAMEs) and fatty alcohols (as TMS derivatives) were determined simultaneously by employing an Agilent HP series GC 6890N coupled with a HP 5973 MS detector (EI, 70 eV), split−splitless injector and an HP 7683 autosampler. An aliquot (1 μL) of each methylated and silylated sample was injected into the gas chromatograph at a split ratio of 1:20. Separation of samples was achieved using an HP-5 MS capillary column (5% phenyl/95% methyl siloxane) that was 30 m in length, 0.25 mm in internal diameter, and had a 250 μm coating thickness. Helium was used as a carrier gas at a flow rate of 0.6 mL/min. The injector and transfer line temperatures were set at 250 and 300 °C, respectively. The oven temperature program was as follows: initial temperature 90 °C and increase to 220 at 3 °C/min, where it was held for 25 min.

Identification of peaks corresponding to FAME was accomplished by means of a standard mixture of 37 FAMEs purchased from Supelco and by reference to the NIST 98 (NIST MS search v6.1d) mass spectra library. The mixed FAME standard furthermore served for the calculation of fatty acid response factors. The calculated response factors were found to range between 0.88 and 1.15, and they were applied to the areas derived from the chromatographic traces. Individual TMS ethers of fatty alcohols were identified using their mass spectra and by reference to NIST 98 (NIST MS search v6.1d) and Wiley 275 (Wiley, New York, NY) mass spectra libraries.

Tocopherol Determination. The analysis and quantification of tocopherols was carried out in portions of total lipid (Folch) extracts that were diluted (10% v/v) in chloroform/methanol (1:1 v/v). An HPLC system (Agilent Technologies, Model 1050) combined with a quaternary pump, autosampler, diode array detector (HP-1050), fluorescence detector (HP-1046A), and data analysis software was used. Aliquots of 20 μL were subjected to reversed phase HPLC analysis with UV (280 and 295 nm) and fluorescence detection (λ_{ex} = 295 nm and λ_{em} = 330 nm) as previously described (*25*). External standard quantification was performed based on a series of five different standard concentrations of α-, γ-, and δ-tocopherol.

Ascorbic Acid Determination. Approximately 100 mg of avgotaracho was thawed and immediately homogenized for 3 min in ice-cold 10% TCA at 9000 rpm with an UltraTurrax T25 (IKA, Werke, Janke and Kunkel GmbH and Co KG, Staufen, Germany) homogenizer and centrifuged for 10 min at 800*g*. The ascorbic acid concentration in the supernatant was measured by the dinitrophenylhydrazine method (*26*).

Cholesterol, Phytosterols, and Squalene Determination. Cholesterol, phytosterols, and squalene were determined in aliquots of the Folch extract (containing 20−30 mg of lipids) by GC/FID after hot saponification followed by methylation with BF_3/MeOH and silylation with BSTFA as was described earlier, except for using 5-α-cholestane (1 mg/mL) as the internal standard instead of methyl heneicosanoate. An Agilent HP series GC 6890 (Avondale, PA) equipped with a flame ionization detector, split−splitless injector, and HP 6890 autosampler was employed. One microliter of each sample and standards were injected in the gas chromatograph at a split ratio of 20:1. Separation of squalene and sterols was achieved on a SGE (Melbourne, Australia) BPX50 capillary column (30 m long, 0.25 mm internal diameter) coated with a 0.25 μm thick film of 50% PH phenylmethylpolysiloxane as previously described (*27*). The identification and quantification of squalene, cholesterol, β-sitosterol, campesterol, and stigmasterol were performed by using standard solutions and by constructing the respective standard curves using 5-α-cholestane as the internal standard. The peak corresponding to Δ^5-avenasterol was recognized as the first major peak eluting after β-sitosterol in chromatograms obtained from virgin sesame oil, which has been reported to be relatively rich in the specific phytosterol and was quantified according to a β-sitosterol reference curve (*27*).

Washed Rabbit Platelet Aggregation Assay. TL, NL, and PL fractions were tested for their biological activity against washed rabbit platelets that were isolated from rabbit blood as described previously (*14*). PAF standard and examined samples were dissolved in a solution of 2.5 mg of BSA per mL of normal saline. Platelet aggregation was measured in a chrono-log aggregometer (model 400-VS) coupled to a chrono-log recorder (Havertown, PA). Various concentrations of the examined sample were added into the aggregometer cuvette, and the aggregation induced by the sample was measured as the percentage of maximum reversible aggregation. The aggregatory activity of the sample was expressed as the amount of sample (in ng per μL of platelet suspension) that is able to induce 50% of the maximum reversible aggregation of the respective sample, namely, 50% efficient concentration (EC_{50}). To study the inhibitory activities of lipid fractions, platelets were preincubated with the samples for 1 min prior to the addition of PAF (2.5×10^{-11} M, final concentration in the cuvette) or thrombin (0.125 U/cuvette). Platelet aggregation induced by PAF or thrombin was measured as PAF or thrombin-induced aggregation before (considered as 0% inhibition) and after the addition of various concentrations of the examined sample. Consequently, the plot of percent inhibition versus different concentrations of the sample was constructed, and from this plot, the concentration of the sample that inhibited 50% PAF or thrombin-induced aggregation (IC_{50}) was calculated. The IC_{50} values are expressed as nanograms of sample per microliter of platelet suspension (*28*).

Table 1. Proximate Composition and Energy Content of Salted and Dried *M. cephalus* Roe

	Greece[a]	Florida	Nagasaki, Japan	Taiwan	Italy
moisture (%)	45.1 ± 3.5	30.5	23.4	25.2	25.2
total lipids (% fw)	17.7 ± 1.5	25.7	33.1	26.2	29.7
crude protein (% fw)	33.6 ± 2.2	35.5	38.7	44.2	42.8
energy (kcal/100 g fw)	313.3 ± 18.2	373.3[b]	452.7[b]	412.6[b]	438.5[b]
ash (% fw)	2.97 ± 0.31	5.4	4.4	2.5	2.7
total lipids/crude protein	0.53 ± 0.03	0.72	0.86	0.59	0.69
ref	present work	*4*	*3*	*3*	*3*

[a] Each value represents the mean ± SD of three individual roe samples, analyzed in triplicate. [b] Calculated; fw: fresh weight.

Indices Calculations. To compare the fatty acids profile of *M. cephalus* roes and other kinds of food, the atherogenic index (AI) and thrombogenic index (TI) (*29*) were calculated, where AI = (12:0 + 14:0 + 16:0)/(ω3PUFA + ω6PUFA + MUFA) and TI = (14:0 + 16:0 + 18:0)/(0.5 MUFA + 0.5 ω6PUFA + 3 ω3PUFA + ω3PUFA/ω6PUFA). For TI and AI calculations, the fatty acid concentrations were expressed as grams per kilogram of total fatty acids.

Statistical Analysis. Chemical and chromatographic analyses of three individual samples were performed in triplicate—except as otherwise indicated. As the coefficients of variation (CV) of the measurements were low, the results presented are the average and standard deviations of all obtained values. Data manipulation was performed by means of Microsoft Excel (Microsoft Corp., Redmond, WA). Hierarchical cluster analysis was carried out by Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Herndon, VA).

RESULTS AND DISCUSSION

Crude Composition and Energy Content. The proximate composition and energy content of waxed salted and dried *M. cephalus* roe are presented in **Table 1**. Greek avgotaracho contained more water (45.1%) as compared to 23.4−25.2 and 30.5% reported for salted and dried mullet roes from Japan, Taiwan, Italy (*3*), and from Florida (*4*), respectively. The obvious reason for this differentiation is that Japanese karasumi and Italian botargo are left to dry after their initial processing (*2, 4*), while Greek avgotaracho is coated with beeswax, which prevents further water loss. As a consequence of the higher water content, Greek avgotaracho contained less protein, total fat, and energy on a fresh weight basis, as compared to the respective products (**Table 1**). The ash content of Greek avgotaracho was within the values reported for similar products (**Table 1**). The protein and fat contribution to the total energy was calculated to be ∼43 and 50%, respectively.

Lipid Classes. Neutral lipids comprised 73.7 ± 1.7% of the avgotaracho total lipid extract (**Table 2**), something expected for lipid-rich fish roe (*30*). Wax and steryl esters predominated

Table 2. Lipid Class Composition[a] (% Total Lipids) of Greek Avgotaracho[b]

lipid content (% wet wt)	17.7 ± 1.5[c]
wax and steryl esters	63.7 ± 1.1[d]
triacylglycerols	3.1 ± 0.9[d]
free fatty acids	3.8 ± 0.7[d]
cholesterol	1.6 ± 0.7[d]
unidentified neutral lipids	1.4 ± 0.2[d]
total neutral lipids	73.7 ± 1.7[e]
phosphatidylethanolamine	5.0 ± 1.2[d]
phosphatidylinositol	0.63 ± 0.11[d]
phosphatidylcholine	20.3 ± 0.7[d]
lysophosphatidylcholine	0.55 ± 0.07[d]
sphingomyelin	0.83 ± 0.09[d]
total polar lipids	27.3 ± 2.8[e]

[a] Data are mean ± SD obtained from lipid extracts of three individual roe samples, analyzed in triplicate. [b] Wax-coated, salted, and dried mullet roe. [c] Determined gravimetrically after Folch extraction. [d] Determined by TLC analysis as % NL and PL fractions, respectively, and converted to % total lipids. [e] Determined gravimetrically after separating TL in NL and PL.

among the lipid classes comprising 63.7 ± 1.1% of avgotaracho lipids (**Table 2**) in agreement with previous studies, which reported wax ester contents as high as 70% of mullet roe lipids, with steryl esters comprising only a small fraction—less than 1%—of total lipids (*3, 6*). Wax esters occur as a major lipid class in several species of marine animals. Their role is unclear, but they could serve for buoyancy, permeability control, insulation, and act as an energy reserve or a fatty acid reserve for modifying structural lipids after egg fertilization (*31*). Contrary to fish oils, which are highly susceptible to oxidation both in living organisms and during processing, marine wax esters have been proven to be remarkably stable such as, for example, during the production of Japanese karasumi (*3*), probably because they are not liquid but rather waxy solids (*2, 32*). Furthermore, marine wax esters represent good sources of ω3 fatty acids (*2, 32*) and have been shown to be rapidly digested and absorbed by rats (*32*).

PC and phosphatidylethanolamine were the main avgotaracho phospholipid classes comprising 70.4 and 18.2% of total phospholipids, respectively (**Table 2**), in agreement with literature data concerning the phospholipid composition of teleost fish eggs (*30, 33*).

Fatty Acid Composition. The fatty acid composition of total (TL), neutral (NL), and polar lipids (PL) of Greek avgotaracho is presented in **Table 3**. In TL, the predominant fatty acids were 16:0, 16:1ω7, 18:1ω9, and 22:6ω3 (DHA). MUFA accounted for 44.6% of total fatty acids, followed by PUFA and SFA, similar to the fatty acid profile reported for salted mullet roes from Florida (*4*), presented also in **Table 3**. HUFA mainly consisted of 20:5ω3 (EPA) and 22:6ω3 (DHA) at a ratio of ~1:2 (**Table 3**), which is typical for fish roe lipids (*7, 30*). The ω3 HUFA content of avgotaracho was 2.02 ± 0.30 g/100 g tissue weight, with 20:5ω3, 22:5ω3, and 22:6ω3 comprising 26, 16, and 58% of ω3 HUFA, respectively. Similar fatty acid profiles were observed in NL, something expected as NL accounted for 73.7 ± 1.7% of TL (**Table 2**). This similarity was confirmed by conducting hierarchical cluster analysis on a matrix of squared Euclidean distances based on the standardized concentrations of TL, NL, PL, and wax ester main fatty acids by using Ward's algorithm, which resulted in the dendrogram presented in **Figure 1**. PL, which comprised 27.3 ± 2.8% of TL, had different fatty acid patterns with ω3 PUFA predominating, followed by SFA and MUFA (**Table 3**), and formed a distinct group in the dendrogram of **Figure 1**. The major fatty acids of PL were 22:6ω3 and 16:0 followed by 18:1ω9 and 20:5ω3 (**Table 3**). The same fatty acids are known to enrich

Table 3. Composition (wt %) of Fatty Acids in TL, NL, and PL of Processed *M. cephalus* Roe

	Greek avgotaracho[a,b]			Florida mullet[c]
fatty acid	TL	NL	PL	TL
		SFA		
14:0	2.77 ± 0.20	2.99 ± 0.34	1.45 ± 0.22	5.6
15:0	0.3 ± 0.03	0.28 ± 0.03	0.26 ± 0.02	1.8
16:0	14.7 ± 0.86	11.7 ± 0.69	19.1 ± 1.13	11.6
17:0	0.6 ± 0.11	0.72 ± 0.07	0.52 ± 0.01	4.5
18:0	3.6 ± 0.11	1.54 ± 0.18	9.12 ± 0.68	4.4
20:0	tr	tr	0.06 ± 0.02	nr
22:0	0.10 ± 0.02	0.12 ± 0.03	tr	nr
23:0	0.16 ± 0.02	0.28 ± 0.12	0.20 ± 0.02	nr
24:0	0.27 ± 0.03	0.36 ± 0.06	0.47 ± 0.04	nr
		MUFA		
15:1	0.05 ± 0.02	0.07 ± 0.02	tr	nr
16:1ω9	0.97 ± 0.06	0.94 ± 0.03	0.55 ± 0.04	nr
16:1ω7	13.6 ± 0.61	15.0 ± 1.22	5.12 ± 0.47	18.1
16:1ω5	0.53 ± 0.07	0.48 ± 0.12	0.36 ± 0.08	nr
17:1	0.39 ± 0.07	0.12 ± 0.05	0.06 ± 0.01	nr
18:1ω9	16.3 ± 0.52	18.0 ± 0.44	8.06 ± 0.43	18.3
18:1ω7	6.55 ± 0.52	7.10 ± 0.46	4.10 ± 0.35	nr
18:1 others	1.30 ± 0.15	1.38 ± 0.18	0.68 ± 0.09	nr
20:1ω9	0.33 ± 0.05	0.41 ± 0.06	0.22 ± 0.03	nr
22:1ω9	3.82 ± 0.42	4.41 ± 0.56	2.44 ± 0.19	nr
24:1ω9	0.27 ± 0.03	0.38 ± 0.04	0.21 ± 0.01	nr
		PUFA		
16:2ω6	0.57 ± 0.10	0.50 ± 0.12	0.28 ± 0.06	nr
18:2ω6	1.90 ± 0.11	2.03 ± 0.14	1.12 ± 0.10	6.1
18:3ω6	0.47 ± 0.04	0.51 ± 0.08	0.39 ± 0.03	nr
18:3ω3	0.76 ± 0.04	0.82 ± 0.01	0.42 ± 0.04	3.4
18:4ω3	5.55 ± 0.61	6.11 ± 0.48	2.70 ± 0.32	4.2
20:2ω6	0.08 ± 0.03	0.10 ± 0.01	0.07 ± 0.01	nr
20:3ω6	0.37 ± 0.02	0.42 ± 0.02	0.37 ± 0.04	nr
20:4ω6	1.65 ± 0.16	0.91 ± 0.10	3.38 ± 0.46	1.6
20:3ω3	0.16 ± 0.01	0.21 ± 0.01	0.07 ± 0.01	nr
20:5ω3	4.90 ± 0.21	4.18 ± 0.16	8.05 ± 0.41	6.4
22:2ω6	0.05 ± 0.01	0.55 ± 0.05	tr	nr
22:4ω6	0.39 ± 0.03	0.56 ± 0.05	0.25 ± 0.08	nr
22:5ω6	0.67 ± 0.08	0.56 ± 0.1	1.17 ± 0.06	nr
22:5ω3	3.10 ± 0.19	3.16 ± 0.31	3.54 ± 0.26	5.0
22:6ω3	11.1 ± 0.33	8.87 ± 0.57	21.3 ± 0.97	8.4
SFA	22.1 ± 1.11	18.1 ± 0.70	31.3 ± 0.71	27.9
MUFA	44.6 ± 1.33	48.7 ± 0.98	22.1 ± 1.21	36.4
PUFA	31.1 ± 0.54	28.4 ± 1.12	42.8 ± 1.71	30.9
ω3	25.5 ± 0.20	23.4 ± 0.74	36.1 ± 1.32	23.2
ω6	5.61 ± 0.22	5.09 ± 0.34	6.7 ± 0.36	7.7
ω3/ω6	4.55 ± 0.26	4.61 ± 0.03	5.4 ± 0.01	3.0
EPA/DHA	0.44 ± 0.03	0.47 ± 0.05	0.38 ± 0.02	0.8

[a] Values are mean ± SD obtained by analyzing extracts from three individual roe samples in triplicate (present work). [b] Wax-coated, salted, and dried mullet roe. [c] Salted and dried mullet roe (*4*); TL: total lipids; NL: neutral lipids; PL: polar lipids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EPA: eicosapentaenoic acid, 20:5ω3; DHA: docosahexaenoic acid, 22:6ω3; tr: traces (<0.05%); and nr: not reported

PC of several salted fish roe products (*33*) and are predictably predominate also in mullet roe PC, which is a major phospholipid class of avgotaracho lipids (**Table 2**).

From the results presented in **Table 3**, it seems that the consistent trends of selective incorporation of fatty acids in different egg lipid classes reported in the literature (*34*) also are followed in the case of mullet roe: MUFAs were predominant in TL and NL, comprising 44.6 and 48.7% of fatty acids, respectively, while PL contained more PUFA (mainly 22:6ω3 and 20:5ω3), more arachidonic acid (20:4ω6), and more SFA than NL. NL are generally regarded primarily as an energy reserve. Therefore, the presence of high amounts of MUFA in the NL fraction of roe lipids is in agreement with the suggestion that these fatty acids are preferred substrates for catabolism during embryonic and larval development (*30, 34, 35*). In

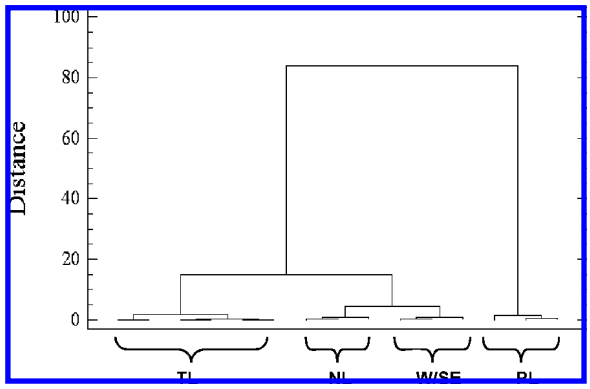

Figure 1. Grouping of selected lipid fractions of waxed salted and dried mullet roe, based on their major fatty acids, namely, 16:0, 16:1, 18:0, 18:1, 20:5ω3, and 22:6ω3. TL: total lipids; NL: neutral lipids; W/SE: wax and steryl esters; and PL: polar lipids.

relation to human nutrition, MUFAs have received increasing attention during recent decades as being potentially beneficial due to their association with low rates of cardiovascular heart disease in olive oil-consuming populations of the Mediterranean basin (*36*).

The fatty acid composition of egg lipids is quite different from those of body oils, indicating selective catabolism of certain fatty acids and/or selective mobilization of other fatty acids from reserves to eggs (*34, 35*). However, as dietary fatty acids have been shown to affect to some extent the fatty acid composition of eggs from farmed mullets (*7*), the agreement of our findings with literature data is noteworthy indicating (a) minimal fatty acid alterations during the production of avgotaracho, something observed also in the case of karasumi (*3*) and (b) similar feeding habits and diet composition of wild striped mullets. One difference among literature data was observed in the odd-numbered fatty acids (15:0, 15:1, 17:0, and 17:1), which were elevated in mullet roes from the Mexico Gulf (Florida) reaching up to 6.3% of total fatty acids (*4*), while in mullet roes from the Mediterranean (present work) and in eggs of mullets reared in sea ponds from Hawaii (Pacific Ocean) (*7*), they were either not detected or did not exceed 1.3% (**Table 3**). This geographical differentiation persisted (a) in the odd-numbered fatty alcohols of mullet roe wax esters (see following section) and (b) in mullet body lipids, with odd-numbered fatty acids ranging between 3.8−20.9% of total fatty acids of striped mullets from the US Atlantic coast (*37, 38*), while being <1.3% of total fatty acids in body lipids of mullets from the Black and Marmara Seas (*39*) or reared in Greece (*40*). It was suggested (*41*) that the origin of the increased concentrations of odd-numbered fatty acids could be the result of either the biosynthesis of such acids at an unusually high rate or their catabolism after ingestion at an unusually low rate. The latter hypothesis is considered as more plausible (*38*) and in agreement with geographical variations observed, which do not support the biosynthesis hypothesis since if the biosynthesis hypothesis was true, the results should be more homogeneous. Therefore, differences in the availability of fatty acids through the trophic chain are more possibly the reason for the observed variations.

Wax Esters of Greek Avgotaracho. A representative total ion chromatogram of fatty acids and fatty alcohols of mullet roe wax and steryl esters is given in **Figure 2**, while the fatty acids and fatty alcohol composition of avgotaracho wax esters are presented in **Tables 4** and **5**, respectively. The distribution of major fatty acids in wax esters is in agreement with literature data (**Table 4**) and is similar to that of NL (see **Table 3**). Such a similarity is not surprising as wax esters are the predominant NL class, and this is depicted in the dendrogram of **Figure 1**, where the fatty acid profiles of TL and NL are grouped together with those of wax esters. MUFA comprised almost 50% of the wax fatty acids, followed by PUFA and SFA. The fatty acid composition of avgotaracho wax esters is similar to that reported for raw mullet roe from Florida (*6*), while it contains less ω3 PUFA and more MUFA than Italian botargo (**Table 4**).

Saturated alcohols (mainly 16:0, 14:0, and 18:0) were the more abundant fatty alcohols in mullet roe wax esters, and along with monounsaturated alcohols comprised the bulk of fatty alcohols (**Table 5**). The same pattern also was followed in mullet roes from Mexico Gulf (*6, 8*) but, surprisingly, was opposite to that of Italian botargo (*2*) where monounsaturated alcohols were reported to predominate (**Table 5**). The geographical differentiation in the odd-numbered fatty acids of mullets from the Mediterranean and U.S. Atlantic coast discussed in the previous section also was observed in the odd-numbered fatty alcohols, which were reported to be as high as 8.0−20.3% in Florida mullet roes (*6, 8*) but were very low in Greek avgotaracho (present work) and not detected in Italian botargo (*2*) (**Table 5**).

Tocopherols and Ascorbic Acid. Greek avgotaracho contained 4.09 ± 0.84 mg/100 g tocopherols (**Table 6**), a value higher than that (1.0 mg/100 g) reported in the USDA Data Bank (*42*) for the flesh of raw mullet. α-Tocopherol comprised 99.5% and γ-tocopherol the rest (0.5%) of total tocopherols. Fish roes are considered to be rich sources of tocopherols, normally containing more vitamins than the respective fish flesh, as α-tocopherol has a specific role in the reproduction of teleosts—acting to stabilize biological membranes and to prevent autoxidation of polyunsaturated fatty acids—and problems of hatchability and embryo survival are known to occur in eggs originating from females fed with diets containing low vitamin E levels (*43*).

The ascorbic acid content of Greek avgotaracho was 26.03 ± 4.1 mg/100 g, within the range of 0.5−45 mg/100 g reported for ovarian tissues of wild and domesticated fish (*44*) and several times higher than the value of 1.2 mg/100 g reported for total ascorbic acid content of raw mullet in the USDA Data Bank (*42*). High ascorbic acid concentrations have been associated with gonad tissues in teleost fishes (*44, 45*). Since ascorbic acid together with α-tocopherol is considered to be critical for the normal development of fish embryos (*45*)—as data obtained from cultured fish studies revealed (*34, 45*)—such a distribution is not surprising.

Cholesterol, Phytosterols, and Squalene. The cholesterol content of avgotaracho was 387.5 ± 10.8 mg/100 g fw, within the range of 260−590 mg/100 g fw reported for several salted fish roe products (*33*) and lower than 425 mg/100 g fw for hen eggs reported in the USDA Data Bank (*42*). Avgotaracho lipids were additionally found to contain certain amounts of phytosterols (β-sitosterol, stigmasterol, campesterol, and Δ^5-avenasterol (**Table 6**)) and squalene at concentrations of 5.4 ± 0.7 mg/100 g, significantly higher than the values of 1.0−1.4 mg/100 g fw reported for Mediterranean fish (*46*). Squalene is widely distributed in nature, existing in large quantities in shark liver oil, while reasonable amounts are found in olive oil, wheat germ oil, and rice bran oil. The squalene content of hen eggs from the Greek market was 0.65 ± 0.15 mg/100 g egg (Kalogeropoulos, N., unpublished data), almost 1 order of magnitude lower than the respective avgotaracho content. As *M. cephalus* is not carnivorous, feeding mainly on organic detritus and diatoms (*47*), and as algal oil contains more phytosterols and squalene than fish oils (*48*), the feeding habits of mullet could provide an explanation for the presence of phytosterols

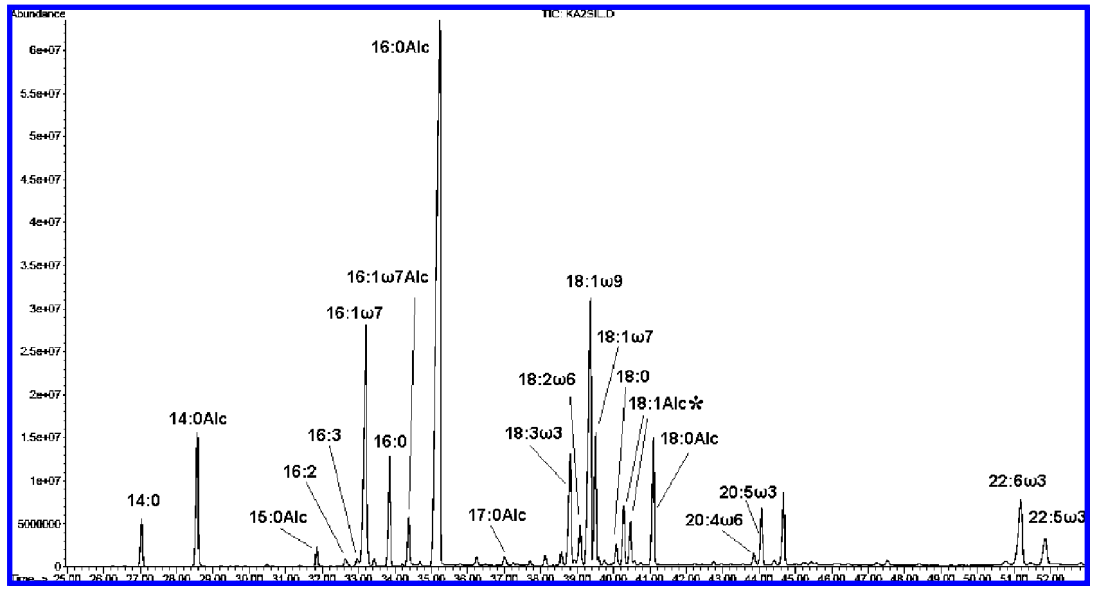

Figure 2. Partial GC/MS total ion chromatogram of fatty acids (as methyl esters) and fatty alcohols (as trimethylsilyl ethers) derived from the wax and steryl ester fractions of *M. cephalus* roe and separated on an HP-5 MS capillary column. Alc: alcohol; *18:1ω9Alc (left peak) and 18:1ω7Alc (right peak).

Table 4. Composition (wt %) of Fatty Acids in Wax and Steryl Ester Fractions Extracted and Purified from Processed and Raw *M. cephalus* Roe

	processed roe		raw roe	
fatty acid	Greek avgotaracho[a]	Italian botargo	Mexico Gulf	Mexico Gulf
14:0	3.40 ± 0.48	1.6	1.0	1.2
15:0	0.20 ± 0.02	nr	0.4	0.5
16:0	7.41 ± 0.47	9.5	4.1	4.4
16:1ω9	0.80 ± 0.05	nr	nr	nr
16:1ω7	19.9 ± 0.82	11.6	23.3	18.5
16:2ω6	0.80 ± 0.16	0.2	4.8	6.8
16:3	0.11 ± 0.07	0.3	2.2	2.6
17:0	0.78 ± 0.04	nr	0.1	0.3
17:1	0.81 ± 0.10	nr	2.2	2.9
18:0	1.37 ± 0.10	1.8	0.8	0.3
18:1ω9	23.3 ± 0.92	15.4	12.8	14.1
18:1ω7	7.78 ± 0.53	nr	nr	nr
18:2ω6	2.84 ± 0.20	1.6	3.8	3.9
18:3ω3	6.1 ± 0.68	0.7	2.2	2.8
18:4ω3	4.5 ± 0.23	0.7	3.1	3
20:1ω9	0.22 ± 0.07	0.4	nr	0.5
20:3ω6	0.33 ± 0.06	0.3	0.4	0.4
20:4ω6	0.87 ± 0.12	2.1	1.3	3.1
20:5ω3	4.36 ± 0.33	10.0	8.8	9.3
22:5ω3	3.09 ± 0.27	4.4	4.4	7.2
22:6ω3	9.40 ± 0.88	23.7	5.9	11.1
SFA	13.2 ± 1.1	12.9	6.4	6.7
MUFA	53.0 ± 1.9	27.4	38.3	36.0
PUFA	32.7 ± 1.3	43.3	33.8	47.2
ω3	27.6 ± 0.9	38.8	21.3	30.4
ω6	4.84 ± 0.12	4.2	10.3	14.2
ω3/ω6	5.85 ± 0.31	9.2	2.1	2.1
EPA/DHA	0.47 ± 0.07	0.4	1.5	0.8
ref	present work	*2*	*5*	*8*

[a] Values represent mean ± SD of data obtained from analyzing wax ester fractions of three individual roe samples in triplicate; other abbreviations are as in **Table 3**.

and increased amounts of squalene in its roe lipids.

AI and TI. The AI and TI of avgotaracho total lipids, presented in **Table 7**, are among the lowest values reported for fish oils and foods including olive oil and several kinds of fish and meat.

Antiplatelet Activity of Mullet Roe Lipids. The lipid fractions (TL, NL, and PL) extracted from three samples of

Table 5. Fatty Alcohols (% Total Fatty Alcohols) in Wax and Steryl Ester Fractions Extracted and Purified from Processed and Raw *M. cephalus* Roe

	processed roe		raw roe	
fatty alcohol	Greek avgotaracho[a,b]	Italian botargo	Mexico Gulf	Mexico Gulf
14:0	10.5 ± 1.21	nr	12.4	9.9−21.7
14:1	0.2 ± 0.02	nr	nr	nr
15:0	1.5 ± 0.20	nr	7.4	6.2−18.6
16:0	65.5 ± 1.36	13.7	46.8	42.0−54.3
16:1	5.1 ± 0.24	66.2	12.9	6.5−14.4
17:0	0.7 ± 0.01	nr	2.1	1.6−1.7
17:1	0.7 ± 0.08	nr	1.3	1.5−2.8
18:0	7.6 ± 0.64	0.8	5.3	2.8−6.4
18:1	7.0 ± 0.83	14.0	5.6	3.3−5.3
18:2	0.1 ± 0.03	nr	1.1	nr
20:0	0.3 ± 0.02	nr	0.2	nr
20:1	0.1 ± 0.03	1.4	0.5	nr
saturated alcohols	85.6 ± 1.55	14.5	74.2	78.7−86.9
monounsaturated alcohols	13.2 ± 1.50	81.6	20.3	13.1−21.1
ref	present work	*2*	*8*	*6*

[a] Values represent mean ± SD of data obtained from analyzing the wax ester fractions of three individual roe samples in triplicate. [b] Wax-coated, salted, and dried mullet roe; nr: not reported.

waxed mullet roe were tested for their ability to either induce platelet aggregation or inhibit PAF (and thrombin-induced platelet aggregation). The biological activity of each lipid fraction along with its corresponding IC_{50} value is shown in **Table 8**. The TL fraction of waxed mullet roe is a potent inhibitor of platelet aggregation, showing a stronger inhibitory activity against PAF-induced aggregation than thrombin-induced aggregation. The IC_{50} value (for PAF-induced aggregation) of TL corresponds to an initial amount of 20 μg of avgotaracho. The inhibitory activity of TL is mainly attributed to their PL content since PL are 3- and 9-fold stronger inhibitors of PAF- and thrombin-induced platelet aggregation, respectively, as compared to NL. PL also show a slight aggregatory activity toward platelets at concentrations 10-fold higher than those showing inhibitory action. The bimodal effect of PL on platelet aggregation can be explained by the fact that the examined lipid fractions are mixtures of lipid molecules that demonstrate either

Table 6. Tocopherols, Ascorbic Acid, Squalene, Cholesterol, and Phytosterols (mg/100 g fw) in Greek Avgotaracho[a]

microconstituent	Greek avgotaracho[b]
tocopherols[c]	4.1 ± 0.84
ascorbic acid	26.0 ± 3.1
squalene	5.4 ± 0.70
cholesterol	387.5 ± 10.8
campesterol	5.1 ± 1.5
stigmasterol	5.4 ± 1.6
β-sitosterol	6.7 ± 1.1
Δ^5-avenasterol	4.5 ± 0.69

[a] Wax-coated, salted, and dried mullet roe. [b] Values represent mean ± SD of three individual roe samples analyzed in duplicate. [c] α-Tocopherol comprised 99.5% of tocopherols.

Table 7. AI and TI Indices in Greek Avgotaracho[a] and Other Kinds of Food

food	AI	TI	ref
Greek avgotaracho[b]	0.26 ± 0.02	0.25 ± 0.02	present work
Mediterranean fish, raw	0.71−1.2	0.30−0.60	*46*
mackerel, raw	0.28	0.16	*29*
olive oil	0.14	0.32	*29*
beef	0.72	0.8−1.4	*29*
pork	0.60	1.4−1.7	*29*
chicken	0.50	0.95	*29*
lamb	1.00	1.3−1.6	*29*

[a] Wax-coated, salted, and dried mullet roe. [b] Values represent mean ± SD obtained from fatty acid analysis of three individual roe samples analyzed in triplicate.

Table 8. Biological Activity of Avgotaracho[a] Lipid Fractions against PAF Inhibition and Thrombin-Induced Platelet Aggregation

lipid fraction	action	aggregation concentrations (ng/μL)	IC_{50} (PAF) (ng/μL)[b]	IC_{50} (thr) (ng/μL)[b]
TL	inhibition		11.4 ± 7.3	138.2 ± 35.8
NL	inhibition		109.3 ± 21.5	123.6 ± 32.3
PL	inhibition/aggregation	140−300	31.7 ± 9.4	14.8 ± 7.1

[a] Wax-coated, salted, and dried mullet roe. [b] Values represent mean ± SD obtained from extracts of three individual roe samples analyzed in triplicate; TL: total lipids; NL: neutral lipids; PL: polar lipids; PAF: platelet activating factor; thr: thrombin; and IC_{50}: sample concentration capable of inducing 50% inhibition of PAF or thrombin-induced aggregation.

aggregatory or inhibitory properties. Contrary to what we observed with the mullet roe lipids, previous studies have shown that lipid fractions obtained from the flesh of several fish (golden trout, rainbow trout, sea bass, and plaice) induce platelet aggregation in vitro (*28*). The main difference between the lipid composition of fish muscle and the lipid composition of fish roe is that fish roe lipids have a higher content of ω3 fatty acids and—in several cases—wax esters. As to whether this compositional difference warrants observed differences of in vitro biological activity remains to be investigated, without overlooking that ω3 fatty acids are moderate inhibitors of in vitro platelet aggregation. Although the structural elucidation and quantitative determination of bioactive lipid molecules is both interesting and challenging, from a nutritional point of view, the overall biological activity of the mixture of biomolecules that is actually consumed seems to be more important.

Nutritional Evaluation. As there are no data concerning the average daily consumption of avgotaracho or similar products in Greece, Europe, or elsewhere, we used a reference unit of 100 g, which is widely used for nutritional evaluation, food labeling, and food composition tables, keeping in mind that as avgotaracho is a rather expensive gourmet product, the usual consumption is not expected to exceed 100 g. The dietary intake of macro- and microconsituents by consuming 100 g of avgotaracho was calculated and compared to typical daily intakes. In the case of fatty acids, data were converted from percent total fatty acids to amounts (g) of individual fatty acids per 100 g of tissue weight, by applying lipid conversion factors for finfish established by USDA researchers (*49*). The results, summarized in **Table 9**, indicate that the consumption of 100 g of avgotaracho could cover 1/3 daily protein intake and almost 1/4 daily fat intake (*50*), providing more MUFA and PUFA than SFA. The intake of ω3 PUFA is expected to be 2.2 times higher than the 900 mg per day recommended by the American Heart Association (AHA) for patients with coronary disease (*51*) and 10 times higher than the 200 mg of marine ω3 PUFA recommended as minimum daily intake by the U.K. Committee on Medical Aspects of Food Policy (COMA) (*52*). The consumption of 100 g of avgotaracho also provides 8.3 g of wax esters and 0.93 g of PC, which exceeds the minimum dietary intake of 0.80 g of PC per day (*53*). Cholesterol intake (387.5 mg) exceeds the recommended daily intake of 230 mg but is still comparable to the cholesterol intake by consuming equal weight of hen egg (calculated to be 427 mg (*42*)).

Table 9. Dietary Evaluation by Consuming 100 g of Greek Avgotaracho[a]

constituent	content per 100 g fw	content per 100 g % daily intake	daily intake	ref
protein (g)	33.7	37	91	*50*
total fat (g)	17.7	27	65	*50*
energy (kcal)	313.3	16	2000	*50*
SFA (g)[b]	2.9	17	17	*50*
MUFA (g)[b]	6.1	25	24	*50*
PUFA (g)[b]	4.0	20	20	*50*
ω3 HUFA (mg)[c]	2020	224	900[d]	*51*
		1010	200[e]	*52*
cholesterol (mg)	387.5	168	230	*50*
α-tocopherol (mg)	4.1	27	15	*56*
ascorbic acid (mg)	26.0	33−39	66−80	*56*
phytosterols (mg)	21.7	9	250	*57*
squalene (mg)	3.8	13	30	*58*
phosphatidylcholine (g)	0.98	122	0.80	*53*
wax esters (g)	8.3			

[a] Wax-coated, salted, and dried mullet roe. [b] Calculated by applying lipid conversion factors for finfish and mollusks (*45*). [c] Determined quantitatively. [d] AHA dietary guidelines for patients with coronary disease. [e] COMA dietary guidelines; abbreviations are as in **Table 3**.

Vitamin E present in 100 g of avgotaracho covers 27% daily intake, which can be regarded as a significant contribution. The presence of relatively high amounts of tocopherols in foods that, like mullet roe, are rich in polyunsaturated fatty acids is beneficial for human consumption, as such unsaturated diets increase the peroxidizability of the lipids and reduce the time required to develop symptoms of vitamin E deficiency, leading to increased requirements for vitamin E to prevent tissue PUFA oxidation. As a result, a ratio of at least 0.6 mg of α-tocopherol equivalent per gram of PUFA is suggested (*54*). According to this ratio, the tocopherol content of avgotaracho (4.09 mg/100 g) is 3.4 times higher than the amount of α-tocopherol required to prevent oxidation of the respective ω3 HUFA, which was determined to be 2.02 g/100 g fw (as described in Fatty Acid Composition). In a recent study, α-tocopherol was suggested to be the most effective antioxidant in reducing oxidant-induced accumulation of free fatty acids in vivo as a result of increased HUFA supplementation (*55*). With regard to ascorbic acid, the consumption of 100 g of avgotaracho covers the 33−39% recommended daily intake (*56*), while the phytosterol and

squalene contribution is expected to be lower (around 10% daily intake *57, 58*) but not negligible. Finally, wax esters provided by 100 g of avgotaracho were calculated to be 8.3 g.

The composition of fish and fish products (especially their lipid content) usually varies, affected by environmental factors, reproductive season, dietary composition, etc. The same is true for mullet, the body lipids of which exhibit seasonal and geographical variations (*38*). However, several reports provide evidence that roe's lipid composition of various fish (although affected by dietary intake) remains relatively constant. This was reported for common food fish (*59*), for wild versus captive white sea bream (*60*) and cod (*61*), while additionally, very small variations were found in crude composition and lipid class composition of salted and dried mullet roe from Nagasaki, Formosa, and Italy (*3*) (see **Table 1**). For the production of Greek avgotaracho, mature mullets are caught from the Ionian Sea in western Greece, during their spawning period and preferably after mid-August. Therefore, the geographical and seasonal variations in crude composition and lipid classes of mullet roe and avgotaracho produced thereinafter are not expected to be enhanced. This is further supported by the fact that the fatty acid composition of avgotaracho NL and PL does not differ significantly from that reported in the literature from other regions. As a result, with the exception of vitamin C, which is very easily oxidized post mortem, the composition of avgotaracho is not expected to be very different from that of processed mullet roes from the Mediterranean (and probably other marine regions affected mainly by moisture content).

The coating of Greek Avgotaracho with beeswax results in a product with higher water content and less fat, protein, and energy content (on a fresh weight basis) as compared to similar products, like Italian botargo and Japanese karasumi. The covering of mullet roe with beeswax appears to be beneficial by many aspects (i.e., maintaining texture and tenderness and pasteurizing the product, as the first wax cover is added at 150 °C) and eliminates contact with air. Additionally, as the lipids and fatty acid composition of avgotaracho exhibited small differences from those of similar products, it seems that wax covering is not likely to significantly alter mullet roe lipids. Thus, it is an attractive process that could be followed by other mullet roe manufacturers, as mullets are very widely distributed and fished or cultivated in many countries (*62*).

According to the data obtained in the present work, Greek avgotaracho should be considered as a high-quality food, providing adequate amounts of protein and fat. Avgotaracho also provides significant amounts of α-tocopherol and ascorbic acid and (to a lesser extent) squalene. The fatty acid profiles of NL and PL as well as the wax ester composition of mullet roe have similarities to those reported from the Mediterranean Sea and differences from the Atlantic coast mullet roe. Avgotaracho lipids are rich in wax esters and PC, showing a beneficial fatty acid profile, as they are rich in MUFA and ω3 HUFA and have low ω6/ω3 ratios. Additionally, total lipids of avgotaracho exhibited a strong inhibition of PAF and thrombin-induced platelet aggregation that was mainly attributed to the PL fraction. The existence of PAF antagonists may provide additional nutritional value to mullet roe products, such as avgotaracho.

Concerning the in vitro antiplatelet activity of avgotaracho, the activity possessed by a food extract in vitro does not necessarily imply that food consumption leads to an attenuated platelet activity in vivo, as the digestive processes and bioavailability of each component finally determine the composition and amount of the in vivo available pool of bioactive constituents. Previous studies showed that the consumption of meals with a strong antiplatelet activity in vitro led to a reduced platelet activity in vivo in humans (*63, 64*). Therefore, the low IC_{50} values of the lipid extracts (corresponding to just a few micrograms of the initial product) in combination with the high amount of polyunsaturated fatty acids and vitamin E provide indications that avgotaracho may merit future nutritional studies focused on its in vivo antiplatelet activity.

ABBREVIATIONS USED

AI, atherogenic index; BSTFA, bis(trimethylsilyl)trifluoroacetamide; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; GC/MS, gas chromatography/mass spectrometry; HPLC, high performance liquid chromatography; HUFA, highly unsaturated fatty acids; MUFA, monounsaturated fatty acids; NL, neutral lipids; PAF, platelet activating factor; PC, phosphatidylcholine; PL, polar lipids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TI, thrombogenic index; TL, total lipids; TLC, thin layer chromatography.

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