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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Fatty Acid Composition and Antioxidant Levels in Muscle Tissue of Different Mediterranean Marine Species of Fish and Shellfish

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The levels of hydrophilic, lipophilic, and enzymatic antioxidants, as well as the fatty acids composition, of triglyceride and phospholipid fractions were determined in the muscle tissue of 21 species of teleosts, 3 species of cephalopods, and 6 species of crustaceans, just caught from the central Tyrrhenian Sea (Mediterranean Sea). The enzymatic activities and the levels of low-molecular-weight antioxidants, and the percentages of fatty acids, showed marked interspecies differences. Our results showed that total polyunsaturated fatty acids (21.7−61.5%) were the highest, followed by saturated (16.9−41.3%) and monounsaturated (9.1−42.8%) fatty acids. The total *n*−3 fatty acids content (16.6−57.1%) was found to be higher than the total *n*−6 fatty acids content (4.1−10.6%). All of the species studied had an *n*−3/*n*−6 ratio of more than 1, confirming the great importance of fish and shellfish as a significant dietary source of *n*−3 polyunsaturated fatty acids and their beneficial role in the Mediterranean type of diet.

KEYWORDS: Tyrrhenian Sea; marine fish and shellfish; water-soluble, lipid-soluble, and enzymatic antioxidants; *n*−3 polyunsaturated fatty acids; Mediterranean diet; HPLC−DAD; GC−MS

INTRODUCTION

n−3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA, C20:5 *n*−3) and docosahexaenoic acid (DHA, C22:6 *n*−3), are believed to have protective action against ischemic heart disease (*1*, *2*). In The Netherlands, consumption of 30 g of fish daily was found to be associated with 50% fewer deaths from coronary heart disease (*2*). In the multiple risk factor intervention trial, cardiovascular mortality was noted to be inversely proportional to the intake of *n*−3 fatty acids over the 10.5 years of followup (*3*). Numerous other trials performed in different populations gave rise to agreeing results. The rarity of ischemic heart disease in Greenland Eskimos may be explained in part by the antithrombotic effect of the long-chained PUFAs, especially EPA, which is prevalent in diets rich in marine oils (*4*). Several studies have shown that dietary *n*−3 PUFAs suppress platelet activating factor (PAF) generation in leukocytes of humans, which is associated with the antagonism of arachidonic acid (AA) metabolism. Tissue arachidonic acid (C20:4 *n*−6) is derived mainly from dietary linoleic acid (C18:2 *n*−6) and is converted into eicosanoids such as prostaglandins and leukotrienes, which act as mediators of inflammation. EPA and DHA are preferred substrates for 5-lipoxygenase as compared with AA; therefore, the substitution of linoleic acid in diets with other PUFAs, such as EPA and DHA, is expected to attenuate inflammatory responses by reducing AA content or inhibiting eicosanoid generation and PAF biosynthesis (*5*).

The fatty acids composition of fish lipids, especially of PUFAs, is species-specific and is correlated to various factors, including dietary, geographic, and environmental factors, reproductive season, fishery period, etc. (*6*). Therefore, when fish consumption is suggested as a means of improving health, both fat content and PUFA composition must be considered. Although it is generally recognized that PUFA composition may vary among species of fish, little attention has been paid to the composition of different species when selecting fish for diet. Thus, this study was carried out to determine the fatty acid composition of different lipid fractions of common marine fish.

In living organisms, oxidative damage to macromolecules is controlled by two types of antioxidant systems. One is represented by enzymes which remove reactive oxygen species, such as superoxide, hydrogen peroxide, and lipid peroxides, and include superoxide dismutase, catalase, and the peroxidases. The other group of antioxidative compounds scavenge free radicals; these compounds are generally of low molecular weight and may be water- or lipid-soluble. Examples of water-soluble free radical scavengers are ascorbate and glutathione, while tocopherol and ubiquinol (reduced coenzyme Q) represent lipid-

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Table 1. Biometric Data of Different Marine Species of Fish and Shellfish Analyzed

class	order	family	species	FAO name	weight (g)	length (cm)
Crustaces	Stomatopoda	Squillidae	*Squilla mantis*	mantis shrimp	30.2 ± 3.5	10.3 ± 1.4
	bivalves	Mytilidae	*Mytilus edulis*	common mussel	5.2 ± 0.6	4.6 ± 0.4
		Ostreidae	*Ostrea edulis*	European flat oyster	7.2 ± 1.5	5.3 ± 0.5
		Veneridae	*Tapes decussatus*	grooved carpet shell	3.6 ± 0.5	2.3 ± 0.2
	Gasteropodes	Buccinidae	*Buccinulum cornea*	striated buccinum	8.1 ± 0.8	3.3 ± 0.4
		Muricidae	*Phyllonotus trunculus*	bondex murex	9.8 ± 1.1	4.4 ± 0.3
Cephalods	Decapoda	Sepiidae	*Sepia officinalis*	common cuttlefish	91.6 ± 11.4	8.0 ± 1.9
		Loliginidae	*Loligo vulgaris*	loligo	36.4 ± 7.5	9.2 ± 2.3
	Octopodidae	Octopodidae	*Octopus vulgaris*	octopus	289.0 ± 32.1	–
Teleosts	Clupeiformes	Clupeidae	*Sardina pilchardus*	sardine	13.0 ± 4.8	10.8 ± 1.5
	Gadiformes	Merluccidae	*Merluccius merluccius*	European hake	75.7 ± 7.9	21.1 ± 2.6
	Perciformes	Serranidae	*Serranus cabrilla*	comber	64.0 ± 11.3	15.0 ± 1.5
		Moronidae	*Dicentrarchus labrax*	sea bass	164.5 ±10.2	20.9 ± 2.8
		Sphyrenidae	*Sphyraena sphyraena*	pike	113.2 ± 24.3	26.7 ± 2.8
		Carangidae	*Trachurus trachurus*	horse mackerel	123.7 ± 19.5	22.5 ± 2.1
		Centracanthidae	*Centracanthus cirrus*	curled picarel	43.0 ± 15.5	13.5 ± 1.6
		Trachinidae	*Trachinus draco*	great weever	43.5 ± 10.3	17.5 ± 1.8
		Mullidae	*Mullus barbatus*	striped mullet	44.2 ± 12.5	14.0 ± 1.4
			Mullus surmuletus	red mullet	64.5 ± 19.7	15.5 ± 1.6
		Sparidae	*Pagellus erythrinus*	common pandora	95.5 ± 21.2	15.5 ± 1.5
			Boops boops	bogue	60.5 ± 19.8	15.3 ± 1.8
			Diplodus annularis	annular seabream	117.7 ± 27.3	16.5 ± 1.9
			Lithognatus marmyrus	striped seabream	132.0 ± 24.5	19.5 ± 2.1
		Scombridae	*Scomber scombrus*	Atlantic mackerel	155.0 ± 26.4	27.3 ± 2.8
			Thunnus alalunga	albacore	250.5 ± 31.2	72.3 ± 5.8
		Cepolidae	*Cepola rubescens*	red band fish	26.5 ± 12.8	22.0 ± 2.1
	Scorpaenifores	Triglidae	*Trigla lyra*	piper gurnad	39.1 ± 16.8	13.7 ± 1.5
			Trigla lucerna	tub gurnad	64.2 ± 22.1	18.5 ± 1.7
			Aspitrigla cuculus	streaked gurnad	32.8 ± 15.9	13.0 ± 0.9
	Pleuronectifomes	Scophthalmiae	*Lepidorhombus boscii*	brill	41.3 ± 16.7	16.2 ± 1.3

soluble low-molecular-weight free radical scavengers. These antioxidants most likely act in a concerted way to protect sensitive molecules such as the unsaturated fatty acids from oxidation during evolution, and this reflects the biochemical and physiological mechanism of adaptation of living organism to oxidative stress induced by environmental factors, including anthropogenic substances (*6*).

Fish contains many oxidation-sensitive substances, such as PUFA. When a fish dies, several oxidative changes occur in the tissues which accelerate the rate of lipid oxidation: the ability to keep the antioxidants in the reduced state diminishes with time because of the loss of reducing compounds, the ability to stabilize lipid free radicals is lost, and lipids will eventually oxidize. In this context, different works compared postmortem quality losses with the rate of change of the antioxidant components (*7*, *8*).

Many investigators have recognized the beneficial role of the Mediterranean type of diet in decreasing the occurrence of cardiovascular diseases (*9*, *10*). In Italy, marine fish and shellfish remain part of the regular diet of a large segment of the population, but the antioxidant levels of common Mediterranean species have not been extensively studied.

The aim of this work was to determine the levels of vitamin E (Vit E), ubiquinols (CoQ_9H_2, $CoQ_{10}H_2$), ubiquinones (CoQ_9, CoQ_{10}), vitamin A (Vit A), reduced and oxidized glutathione (GSH and GSSG), and vitamin C (Vit C); the glutathione peroxidase (GPx), catalase (CAT), and Cu−Zn superoxide dismutase (SOD) activities; and the fatty acids composition of triglyceride and phospholipid fractions in the white muscle tissues of 21 species of teleosts, 3 species of cephalopods, and 6 species of crustaceans (see **Table 1**), just caught from the central Tyrrhenian Sea. These fish are the most common fish caught and consumed annually in Italy.

MATERIALS AND METHODS

Standards and Solvents. Glutathione peroxidase (GPx) (680 units/mg of protein), reduced glutathione (GSH), oxidized glutathione (GSSG), catalase (CAT), and all of the pure standards were purchased from Sigma Chemicals (Milan, Italy). All organic solvents were products of Carlo Erba, Milano (Italy). Water was purified by a Milli-Q_{plus} system from Millipore (Milford, MA).

Materials. Fish and shellfish, listed in **Table 1**, were caught by trawling in the central Tyrrhenian Sea (Anzio, Rome, Italy), sampled directly on-board, and stored in dry ice while being transferred to the laboratory within 8 h. Edible tissue from shellfish and white muscle tissue portions of teleosts, excised by a scalpel from the central part of the dorsal musculature, were divided into approximately 1-g aliquots and deep-frozen at −80 °C until analyzed.

Enzyme Assay. Each sample of muscle (500 mg) was homogenized under a flow of nitrogen for 3 min with a Teflon homogenizer at the maximum speed in the presence of 3.0 mL of 0.01 M PBS, containing 1 mM EDTA, pH 7.0. Homogenates were centrifuged at 100000*g* for 30 min at 4 °C. The supernatant fraction (assay solution) was collected for enzyme assay for reduced and oxidized glutathione and vitamin C.

GPx was determined according to the method of Paglia and Valentine (*11*), which couples hydrogen peroxide reduction to oxidation of NADPH by glutathione reductase. An enzyme unit was defined as that activity which oxidized 1 μmol of NADPH per minute.

The Cu,Zn−superoxide dismutase (SOD) activity was measured by using a RANSOD kit (RANDOX, Grumlin, U.K.); measurements were performed according to the supplier's directions.

CAT activity was assayed according to the method of Aebi (*12*) on 500 μL of assay solution. One CAT unit is defined as the enzyme activity necessary to convert 1 μmol of H_2O_2 to $H_2O + O_2$ at 25 °C and pH 7 in 1 min.

Glutathione Analysis. GSH and GSSG in muscle tissue were assayed by the HPLC method of Reed et al. (*13*). Five hundred microliters of assay solution was added to 10 μg of γ-L-glutamyl glutamic acid (reference standard) and 400 μL of 50% metaphosphoric acid. The dinitrophenyl derivatives were separated and measured using a gradient HPLC system (10 A VP Shimadzu liquid chromatograph)

Table 2. Total Lipids (TL) and Trygliceride (TG) and Phospholipid (PL) Fractions (% of TL) in Muscle Tissue of Different Mediterranean Marine Species of Fish and Shellfish[a]

species	Italian name	TL	TG	PL
Squilla mantis	pannocchia	1.84 ± 0.22	47.8 ± 1.22	17.8 ± 0.22
Mytilus edulis	cozza	1.62 ± 0.36	42.9 ± 1.29	22.3 ± 0.63
Ostrea edulis	ostrica	1.26 ± 0.28	37.3 ± 0.52	20.3 ± 0.41
Tapes decussatus	vongola	1.67 ± 0.24	32.5 ± 0.61	20.2 ± 0.32
Buccinulum cornea	lumachina	1.71 ± 0.41	39.3 ± 1.02	18.8 ± 0.22
Phyllonotus trunculus	murices	1.42 ± 0.33	38.5 ± 1.22	17.1 ± 0.30
Sepia officinalis	sepia	1.42 ± 0.33	23.6 ± 0.22	39.7 ± 0.62
Loligo vulgaris	calamaro	2.41 ± 0.44	23.0 ± 0.63	39.5 ± 0.50
Octopus vulgaris	polpo	2.56 ± 0.37	32.2 ± 0.64	38.1 ± 0.72
Sardina pilchardus	sardina	4.81 ± 0.73	40.8 ± 1.03	18.4 ± 0.31
Merluccius merluccius	nasello	1.23 ± 0.28	33.3 ± 1.08	19.8 ± 0.34
Serranus cabrilla	cerchia	1.92 ± 0.27	31.6 ± 0.66	22.2 ± 0.27
Dicentrarchus labrax	spigola	2.17 ± 0.33	44.6 ± 1.10	17.1 ± 0.20
Sphyraena sphyraena	luccio di mare	0.85 ± 0.11	53.6 ± 1.32	13.3 ± 0.19
Trachurus trachurus	suro	1.97 ± 0.32	39.9 ± 1.74	17.2 ± 0.38
Centracanthus cirrus	zerro	2.16 ± 0.31	46.5 ± 1.22	15.1 ± 0.44
Trachinus draco	tracina	1.65 ± 0.25	40.5 ± 1.37	20.4 ± 0.52
Mullus barbatus	triglia di fango	2.77 ± 0.24	44.7 ± 1.17	16.3 ± 0.29
Mullus surmuletus	triglia di scoglio	3.04 ± 0.88	45.0 ± 1.90	18.1 ± 0.62
Pagellus erythrinus	pagello	1.59 ± 0.18	51.1 ± 1.99	18.2 ± 0.56
Boops boops	boga	2.04 ± 0.25	44.3 ± 1.21	20.1 ± 0.70
Diplodus annularis	sparaglione	2.32 ± 0.31	53.7 ± 1.02	16.5 ± 0.37
Lithognatus marmyrus	marmora	2.19 ± 0.40	47.5 ± 1.88	20.9 ± 0.46
Scomber scombrus	sgombro	5.83 ± 0.81	43.4 ± 1.25	21.7 ± 0.58
Thunnus alalunga	tonno	4.73 ± 066	43.3 ± 1.23	25.8 ± 0.22
Cepola rubescens	cepola	2.67 ± 0.31	54.2 ± 1.22	18.3 ± 0.37
Trigla lyra	cappone	1.94 ± 0.23	46.5 ± 1.40	18.6 ± 0.50
Trigla lucerna	gallinella	1.65 ± 0.23	43.1 ± 1.35	22.4 ± 0.20
Aspitrigla cuculus	coccio	2.63 ± 0.41	55.3 ± 1.12	19.2 ± 0.21
Lepidorhombus boscii	rombo	2.17 ± 0.20	54.7 ± 1.62	15.8 ± 0.22

[a] Each result represents the mean ± SD of five samples for each species.

equipped with an analytical Supelcosil NH_2 column (25 cm × 4.6 mm, 5 μm, Supelco) and a photodiode array detector set at 350 nm (SPD-M, Shimadzu). Mobil phase A was 80% methanol; mobile phase B contained 2 volumes of sodium acetate stock solution with 8 volumes of 80% methanol. The gradient was 80% A/20% B for 10 min, followed by 30 min linear gradient to 100% B at a flow rate of 1 mL/min. GSH and GSSG were quantified by comparison of areas to those of authentic standards, including the reference standard.

Ascorbic and Dehydroascorbic Acid Analysis. Five hundred microliters of assay solution was treated with 2 μg of hypoxanthine (reference standard) and 2 volumes of 2% metaphosphoric acid for Vit C analysis (a) and with 2 volumes of 2% metaphosphoric acid supplemented with 6 mg/mL dithiothreitol for total Vit C (ascorbic + dehydroascorbic acids) analysis (b). Both samples were stored at −80 °C under argon and centrifuged before HPLC analysis. The supernatant was collected and the volume adjusted to 1 mL with water. To determine total Vit C content, the supernatant containing dithiothreitol was incubated at 45 °C for 2 h prior to HPLC analysis. Samples of 50 μL were injected into a Shimadzu liquid chromatograph on an analytical Supelcosil LC-18-DB column (24 cm × 4.6 mm, 5 μm, Supelco) plus its guard column, by using in-line both a photodiode array detector set at 265 nm and an ESA CoulArray (Bedford, MA; oxidation potential, +400 mV). The mobile phase consisted of 0.02 M NaH_2PO_4/CH_3CN (99.5/0.5 v/v), containing 0.6 g/L metaphosphoric acid; flow, 0.6 mL/min. Ascorbic acid was quantified by comparison of areas to those of authentic standards, including the reference standard.

Extraction Procedure for α-Tocopherol, Ubiquinol, and Ubiquinone. Lipid-soluble antioxidants were extracted using the procedure of Burton et al. (*14*).

Determination of CoQ_{10}-H_2/CoQ_{10} and CoQ_9-H_2/CoQ_9. Muscle ubiquinol and ubiquinone were quantified simultaneously by a 10 A VP Shimadzu liquid chromatograph on an analytical Supelcosil LP-18 column (24 cm × 4.6 mm, 5 μm, Supelco) plus its guard column, by using in-line both a photodiode array (SPD-M, Shimadzu) and electrochemical detectors, as previously reported (*15*). The photodiode array detector was set at 275 nm. The electrochemical detection was accomplished by using an ESA CoulArray, which allows the postcolumn electrochemical reduction of ubiquinone to ubiquinol (reduction potential, −600 mV) and the quantitation of ubiquinol with high sensitivity and selectivity (oxidation potential, +600 mV). The mobile phase consisted of 50 mM sodium perchlorate in methanol/2-propanol (55/45 v/v), at a flow rate of 0.7 mL/min. The injection volume of the samples was 10 μL.

Determination of α-, β-, δ-, and γ-Tocopherol. Analyses were performed by GC−MS (Shimadzu MS-QP5050) in SIM mode. Conditions: column, DB1 J&W (25 m × 0.2 mm × 0.33 μm); injection, 1 μL; split ratio, 2; oven temperature, 100 °C for 1 min, to 280 °C at 30 °C/min, and hold for 25 min; injector temperature, 250 °C; carrier gas, helium; flow, 1 mL/min. An electron impact of 70 eV was used for ionization of the compounds; the following ions were monitored: methoxy-δ-tocopherol (*m*/*z* 151, 191, 417); δ-tocopherol-TMS (*m*/*z* 209, 249, 475); γ-tocopherol-TMS (*m*/*z* 223, 489); α-tocopherol-TMS (*m*/*z* 237, 503). Tocopherols were quantitated by comparison of areas to those of authentic standards, including the reference standard.

Determination of Vit A and β-Carotene. Vit A and β-carotene were extracted as previously reported (*16*) and assayed by a Shimadzu liquid chromatograph on an analytical Restek LC-18 ODS amine column (24 cm × 4.6 mm, 5 μm) plus its guard column, by using a photodiode array detector set at 325 nm (Vit A) and 453 nm (β-carotene). Mobile phase A contained 20 μm $NaClO_4$ in MeOH/H_2O (96/4 v/v); mobile phase B contained MeOH/2-propanol (55/45 v/v). The gradient program was 5% B for 5 min, to 20%B in 15 min, and then 90% B in 25 min; flow, 1 mL/min. Retinyl acetate (20 μg) was used as the standard.

Quantification of Triglyceride and Phospholipid Fractions and Their Fatty Acid Patterns. Following extraction of fat from 500 mg of muscle sample, according to the procedure of Bligh and Dyer (*17*), quantification of triglyceride and phospholipid fractions and their fatty acid patterns was performed according to Passi et al. (*18*). TG-FA and PL-FA were analyzed by capillary gas chromatography−mass spectrometry (GC−MS, Shimadzu MS-QP5050) (*18*).

Table 3. Fatty Acids Levels[a] of Triglyceride (TG) and Phospholipid (PL) Fractions in the Muscle Tissue of Different Mediterranean Marine Species of Fish and Shellfish

	Sq. manta		*My. edulis*		*Os. edulis*		*T. decussatus*		*Bu. cornea*		*Ph. trunculus*	
fatty acid	TG	PL	TG	PL	TG	PL	TG	PL	TG	PL	TG	PL
C14:0	5.4	1.2	2.9	0.5	2.4	1.6	1.9	1.0	0.8	1.3	2.5	1.3
C15:0	2.7	1.2	0.4	0.4	0.6	0.8	0.7	1.4	0.5	0.4	0.7	0.4
C16:0	22.2	14.6	11.9	9.7	10.5	11.7	10.6	8.6	12.2	10.8	7.4	8.0
C17:0	1.7	0.9	0.7	0.4	1.2	0.3	0.7	1.8	0.8	0.8	1.1	0.8
C18:0	7.1	9.7	3.8	3.9	6.2	7.5	6.3	6.5	5.5	4.9	4.6	7.4
C20.0	0.6	0.0	0.0	0.0	0.7	0.5	0.5	0.7	0.0	0.7	0.4	1.5
C24:0	1.6	1.9	0.5	2.0	1.4	0.3	1.8	0.5	1.2	0.3	2.2	0.7
∑ saturated	**41.3**	**29.5**	**20.2**	**16.9**	**23.0**	**22.8**	**22.5**	**20.5**	**21.0**	**19.2**	**18.9**	**20.1**
C16:1	9.6	6.4	6.3	0.7	1.8	1.4	0.9	0.7	2.6	1.0	5.0	0.4
C17:1	1.3	1.6	0.6	0.5	0.4	0.2	0.1	0.7	1.4	0.3	1.3	1.1
C18:1[b]	12.4	15.4	5.4	6.2	12.8	10.7	10.7	9.9	8.5	6.5	10.3	7.9
C20:1[c]	4.8	3.3	4.4	6.1	7.1	8.1	3.3	5.5	6.4	9.2	9.9	4.8
C22:1	1.7	0.5	1.6	1.1	2.8	1.7	2.9	1.3	0.6	0.6	2.7	1.0
∑ monoenoic	**29.8**	**27.2**	**18.3**	**14.6**	**24.9**	**22.1**	**17.9**	**18.1**	**19.5**	**17.8**	**29.2**	**15.2**
C18:2 *n*–6	2.1	1.7	1.5	1.2	2.0	2.6	2.0	1.3	2.5	2.4	1.4	2.5
C20:2 *n*–6	1.1	1.5	0.3	1.2	0.4	0.2	0.8	0.8	0.4	1.0	0.9	0.2
C20:3 *n*–6	0.0	0.0	0.4	2.8	1.1	1.4	2.7	2.6	1.6	0.8	1.0	2.0
C20:4 *n*–6	1.3	1.9	1.6	2.0	1.9	1.9	2.4	2.4	2.3	2.1	2.1	2.1
C22:4 *n*–6	1.8	1.9	0.2	2.4	2.3	0.6	2.2	1.9	1.1	1.4	0.6	1.0
C22:5 *n*–6	0.9	1.8	0.4	1.0	1.4	0.7	0.4	0.6	1.0	1.1	1.3	0.4
∑ PUFA *n*–6	**7.2**	**8.8**	**4.4**	**10.6**	**9.1**	**7.4**	**10.5**	**9.6**	**8.9**	**8.8**	**7.3**	**8.2**
C18:3 *n*–3	0.6	1.4	1.9	1.2	1.2	0.7	1.1	0.8	2.0	1.5	0.6	1.1
C18:4 *n*–3	0.2	0.1	1.8	0.0	0.9	0.2	0.5	0.0	1.7	0.3	1.0	0.5
C20:3 *n*–3	0.2	0.0	0.3	0.2	0.6	0.2	1.4	1.4	0.5	0.9	1.9	1.1
C20:4 *n*–3	0.2	0.2	0.1	0.3	0.4	0.3	0.5	0.6	0.3	0.7	0.7	0.8
C20:5 *n*–3	8.9	14.8	28.9	16.0	17.2	15.4	22.8	18.6	23.2	21.8	14.4	25.2
C22:3 *n*–3	0.3	0.6	0.9	1.6	0.9	1.1	0.7	1.8	0.4	1.7	1.2	2.6
C22:5 *n*–3	2.2	2.5	1.4	4.6	2.5	3.2	1.6	3.9	1.7	2.2	2.9	1.2
C22:6 *n*–3	9.1	14.9	21.8	34.0	19.3	26.6	20.5	24.6	20.8	25.1	21.9	24.0
∑ PUFA *n*–3	**21.7**	**34.5**	**57.1**	**57.9**	**43.0**	**47.7**	**49.1**	**51.8**	**50.6**	**54.2**	**44.6**	**56.5**
∑ PUFA	**28.9**	**43.3**	**61.5**	**68.5**	**52.1**	**55.1**	**59.6**	**61.4**	**59.5**	**63.0**	**51.9**	**64.7**
n–3/*n*–6	**3.0**	**3.9**	**12.9**	**5.5**	**4.7**	**6.4**	**4.6**	**5.4**	**5.7**	**6.2**	**7.2**	**6.9**

	Sep. officinalis		*Lo. vulgaris*		*Oc. vulgaris*		*Sa. pilchardus*		*Me. merluccius*		*Ser. cabrilla*	
fatty acid	TG	PL	TG	PL	TG	PL	TG	PL	TG	PL	TG	PL
C14:0	2.1	1.3	5.0	2.0	2.2	1.2	6.5	1.8	4.8	2.1	5.9	1.5
C15:0	1.6	0.9	1.4	0.6	1.7	0.3	1.3	0.7	3.1	1.1	1.2	0.4
C16:0	19.4	16.6	21.3	22.2	19.6	19.9	18.1	20.9	19.6	20.4	16.3	15.3
C17:0	2.2	2.1	2.0	0.5	1.2	1.2	1.5	0.9	2.5	2.5	1.1	0.8
C18:0	8.0	10.0	8.4	6.7	10.5	8.5	5.3	9.9	6.3	10.0	6.9	10.6
C20.0	0.0	0.7	0.0	0.0	0.0	0.0	0.5	0.4	2.8	1.0	0.6	0.5
C24:0	1.0	1.1	0.5	0.7	1.6	0.6	1.0	1.0	0.6	0.3	1.1	1.9
∑ saturated	**34.3**	**32.7**	**38.6**	**32.7**	**36.8**	**31.7**	**34.2**	**35.6**	**39.7**	**37.4**	**33.1**	**31.0**
C16:1	2.7	1.2	4.5	1.1	7.6	2.0	7.4	0.9	8.3	6.0	7.6	3.6
C17:1	0.1	0.0	2.5	1.6	2.5	0.6	1.2	1.1	1.5	1.7	1.1	0.9
C18:1[b]	12.2	8.1	12.1	9.2	19.1	8.1	14.3	10.4	19.6	13.8	22.6	14.6
C20:1[c]	4.0	4.2	2.9	3.1	3.4	2.6	2.6	1.8	2.1	3.9	1.7	3.0
C22:1	0.8	0.8	0.4	0.5	1.2	0.2	0.9	0.6	1.0	1.0	1.5	1.2
∑ monoenoic	**19.8**	**14.3**	**22.4**	**15.5**	**33.8**	**13.5**	**26.4**	**14.8**	**32.5**	**26.4**	**34.5**	**23.3**
C18:2 *n*–6	3.3	1.2	2.4	2.0	1.5	1.1	2.2	1.5	2.5	4.3	1.9	1.8
C20:2 *n*–6	0.3	0.0	0.2	0.0	0.0	0.0	0.9	0.6	0.0	0.9	0.5	0.8
C20:3 *n*–6	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0
C20:4 *n*–6	1.9	1.9	1.5	1.6	1.1	1.9	1.7	1.4	1.2	0.8	1.3	0.8
C22:4 *n*–6	0.0	0.4	0.0	0.0	1.4	1.0	0.0	0.2	0.0	0.1	0.5	1.2
C220:5 *n*–6	1.0	0.7	0.5	0.5	0.8	0.5	0.7	0.8	0.4	0.7	0.9	1.0
∑ PUFA *n*–6	**6.8**	**4.3**	**4.6**	**4.1**	**4.8**	**4.5**	**5.5**	**4.5**	**4.1**	**7.4**	**5.1**	**5.6**
C18:3 *n*–3	0.9	0.5	0.5	0.2	0.7	2.3	1.0	0.7	1.2	1.2	0.8	0.6
C18:4 *n*–3	0.0	0.5	0.0	0.0	0.2	0.4	1.2	0.9	0.1	1.3	1.2	0.4
C20:3 *n*–3	1.5	0.8	0.2	0.1	1.2	1.5	0.0	1.9	0.6	0.0	0.0	3.0
C20:4 *n*–3	0.1	0.0	2.0	0.0	1.8	0.0	0.1	0.4	0.1	0.1	0.6	0.6
C20:5 *n*–3	16.3	21.2	14.1	21.8	9.6	7.7	10.7	5.0	7.9	10.0	8.3	7.5
C22:3 *n*–3	0.0	0.5	0.0	0.0	0.7	1.0	1.3	0.8	0.1	0.5	0.5	0.7
C22:5 *n*–3	0.2	0.8	0.1	0.7	0.4	1.5	0.4	1.6	0.1	0.3	0.9	1.5
C22:6 *n*–3	20.1	24.8	17.5	25.6	10.0	35.9	19.2	33.8	13.6	15.4	15.0	25.8
∑ PUFA *n*–3	**39.1**	**48.7**	**34.4**	**47.7**	**24.6**	**50.3**	**33.9**	**45.1**	**23.7**	**28.8**	**27.3**	**40.1**
∑ PUFA	**45.9**	**53.0**	**39.0**	**51.8**	**29.4**	**54.8**	**39.4**	**49.6**	**27.8**	**36.2**	**34.7**	**45.7**
n–3/*n*–6	**5.7**	**11.3**	**7.5**	**11.6**	**5.1**	**11.2**	**6.2**	**10.0**	**5.8**	**3.9**	**5.3**	**7.2**

Table 3 (Continued)

	Dic. labrax		*Sp. sphyraena*		*Trachu. trachurus*		*Cen. cirrus*		*Trachi. draco*		*Mu. barbaratus*	
fatty acid	TG	PL	TG	PL	TG	PL	TG	PL	TG	PL	TG	PL
C14:0	1.2	0.8	2.3	1.9	3.2	0.9	4.7	2.9	3.1	0.0	5.7	1.3
C15:0	1.7	0.4	1.1	0.6	1.5	0.5	1.5	2.9	1.2	0.0	2.8	1.3
C16:0	18.9	16.9	10.5	21.0	21.5	17.2	15.4	19.5	17.8	14.2	17.0	18.0
C17:0	2.4	2.0	1.7	1.1	0.9	0.7	0.4	1.5	1.0	0.5	1.7	1.0
C18:0	7.7	7.3	9.9	13.9	9.2	13.5	11.8	9.2	8.8	9.8	4.9	10.7
C20.0	0.6	0.4	0.4	0.0	0.5	0.5	0.8	0.0	0.7	0.0	0.6	0.5
C24:0	0.0	0.8	1.3	1.2	0.5	1.7	2.3	1.1	2.3	1.8	1.6	1.5
∑ saturated	**32.6**	**26.6**	**27.9**	**39.7**	**37.3**	**35.0**	**37.3**	**37.4**	**35.5**	**26.3**	**34.4**	**33.0**
C16:1	4.3	2.8	5.2	4.1	6.4	2.0	8.0	5.5	7.4	2.3	6.9	2.5
C17:1	2.2	0.7	1.5	1.0	1.6	1.1	2.4	1.6	1.5	0.0	1.9	1.2
C18:1[b]	17.5	11.6	18.6	4.0	19.2	13.1	24.4	19.2	20.3	14.1	24.1	13.3
C20:1[c]	5.9	2.4	2.7	0.0	2.9	0.9	5.1	1.4	4.1	1.7	0.5	0.8
C22:1	2.4	0.6	0.6	0.0	1.5	0.8	1.1	0.5	1.3	0.8	1.2	0.8
∑ monoenoic	**32.2**	**18.1**	**28.6**	**9.1**	**31.6**	**17.9**	**41.0**	**28.2**	**34.6**	**18.9**	**34.6**	**18.6**
C18:2 *n*–6	3.3	2.3	2.9	2.4	1.6	1.7	1.3	2.7	1.6	1.6	1.7	3.4
C20:2 *n*–6	0.5	1.1	0.5	1.3	0.6	0.5	0.6	0.4	0.7	0.0	0.3	0.5
C20:3 *n*–6	0.2	0.5	0.4	0.0	0.0	0.4	0.3	0.0	0.0	0.0	0.0	0.0
C20:4 *n*–6	0.5	1.3	1.6	1.9	1.2	2.0	1.0	1.0	1.4	2.1	1.1	1.5
C22:4 *n*–6	0.9	0.7	0.3	0.0	0.7	0.9	0.9	0.6	1.1	0.0	0.7	0.9
C22:5 *n*–6	1.0	0.6	0.9	0.8	1.4	1.0	1.0	0.7	1.6	1.2	0.8	0.6
∑ PUFA *n*–6	**6.4**	**6.5**	**6.6**	**6.4**	**5.5**	**6.5**	**5.1**	**5.4**	**6.4**	**4.9**	**4.6**	**6.9**
C18:3 *n*–3	1.9	1.2	1.6	0.7	1.7	1.9	0.6	0.8	0.7	0.6	1.3	1.2
C18:4 *n*–3	0.9	0.6	1.4	0.6	0.7	0.5	0.9	1.9	1.5	0.4	1.2	0.9
C20:3 *n*–3	0.3	0.1	0.2	0.0	0.0	0.6	0.6	0.3	0.1	0.6	0.4	0.6
C20:4 *n*–3	1.0	0.0	1.0	0.0	0.5	0.2	0.0	0.2	0.0	1.0	1.3	1.0
C20:5 *n*–3	8.9	12.4	9.3	9.8	6.8	7.0	7.8	7.0	8.3	11.0	6.4	9.4
C22:3 *n*–3	0.4	0.2	0.4	0.0	0.5	0.7	0.4	0.6	0.3	0.3	0.4	1.1
C22:5 *n*–3	1.5	0.8	1.5	2.1	0.9	1.1	0.6	2.0	1.1	1.0	2.4	1.5
C22:6 *n*–3	13.8	33.5	22.1	31.6	14.5	28.6	5.7	16.2	11.5	35.0	13.0	25.8
∑ PUFA *n*–3	**28.7**	**48.8**	**36.9**	**44.8**	**25.6**	**40.6**	**16.6**	**29.0**	**23.5**	**49.9**	**26.4**	**41.5**
∑ PUFA	**35.1**	**55.3**	**43.5**	**51.2**	**31.1**	**47.1**	**21.7**	**34.4**	**29.9**	**54.8**	**31.0**	**48.4**
n–3/*n*–6	**4.5**	**7.5**	**6.0**	**7.0**	**4.6**	**6.2**	**3.2**	**5.4**	**3.7**	**10.2**	**5.7**	**6.0**

	Mu. surmuletus		*Pa. erythrinus*		*B. boops*		*Dip. annularis*		*Li. marmyrus*		*Sc. scombrus*	
fatty acid	TG	PL	TG	PL	TG	PL	TG	PL	TG	PL	TG	PL
C14:0	3.3	1.6	2.4	1.0	3.2	0.4	3.9	0.5	3.6	1.2	3.9	0.3
C15:0	3.0	1.1	2.2	0.7	1.6	0.0	0.9	0.4	1.5	0.8	1.3	0.3
C16:0	16.5	21.4	13.4	17.0	19.3	15.7	20.3	18.3	20.3	19.2	18.7	11.3
C17:0	1.0	1.1	0.9	1.5	0.6	1.0	0.8	1.1	1.9	1.2	0.8	1.3
C18:0	8.6	12.8	11.1	11.0	8.0	12.3	7.2	12.1	8.8	12.3	7.4	13.3
C20.0	0.8	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.6	0.0	0.0	0.0
C24:0	0.9	1.6	2.0	1.3	1.2	0.8	1.0	2.4	0.8	2.2	0.8	0.6
∑ saturated	**34.1**	**39.6**	**32.0**	**32.5**	**34.7**	**30.2**	**34.1**	**34.8**	**38.1**	**36.9**	**32.9**	**27.1**
C16:1	8.4	1.4	6.3	2.8	6.9	1.3	8.1	2.8	8.2	2.7	10.2	0.8
C17:1	3.1	1.5	2.5	1.1	2.3	0.9	0.9	1.2	1.6	1.1	0.9	1.0
C18:1[b]	25.5	9.1	22.1	11.9	25.7	12.1	23.9	11.4	20.1	12.9	23.9	7.9
C20:1[c]	4.7	1.0	6.6	1.4	3.4	0.6	3.6	0.8	5.2	0.9	3.7	2.6
C22:1	1.1	0.3	1.2	0.2	1.4	0.5	1.1	0.5	0.5	0.9	0.9	0.4
∑ monoenoic	**42.8**	**13.3**	**38.7**	**17.4**	**39.7**	**15.4**	**37.6**	**16.7**	**35.6**	**18.5**	**39.6**	**12.7**
C18:2 *n*–6	1.3	2.9	2.0	3.0	3.5	5.0	0.9	2.0	1.7	1.9	1.4	2.7
C20:2 *n*–6	0.6	1.1	1.0	0.0	0.7	0.0	0.7	0.0	1.0	0.0	0.7	1.0
C20:3 *n*–6	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 *n*–6	2.5	2.1	1.7	2.0	2.1	2.3	1.8	2.1	2.5	2.1	1.8	1.7
C22:4 *n*–6	1.5	2.3	1.4	1.6	0.0	0.0	1.0	2.0	1.5	1.3	1.1	0.6
C220:5 *n*–6	1.0	1.9	2.7	1.7	1.2	1.2	0.9	1.7	1.7	1.6	1.3	0.6
∑ PUFA *n*–6	**7.3**	**10.3**	**8.8**	**8.3**	**7.5**	**8.5**	**5.3**	**7.8**	**8.4**	**6.9**	**6.3**	**6.6**
C18:3 *n*–3	0.8	0.6	0.7	0.6	0.8	4.0	1.1	1.3	1.1	1.1	0.5	1.9
C18:4 *n*–3	0.9	0.0	0.5	0.0	0.7	0.1	0.0	0.1	0.9	0.3	0.4	0.7
C20:3 *n*–3	0.2	0.3	0.2	0.2	0.0	0.4	0.0	0.1	0.1	0.2	0.0	0.0
C20:4 *n*–3	0.7	0.0	0.0	0.0	1.8	0.2	0.6	0.5	1.7	0.2	0.0	1.0
C20:5 *n*–3	6.2	10.9	10.8	14.0	6.8	5.6	6.9	12.6	8.2	8.9	4.2	12.7
C22:3 *n*–3	0.3	0.1	0.2	0.1	0.0	0.3	0.2	0.0	0.0	0.0	0.0	0.5
C22:5 *n*–3	1.0	2.8	0.6	2.0	0.0	2.0	0.0	2.7	0.9	2.1	1.5	3.1
C22:6 *n*–3	5.7	22.1	7.5	24.9	7.8	33.3	14.2	23.4	5.0	24.9	14.6	33.7
∑ PUFA *n*–3	**15.8**	**36.8**	**20.5**	**41.8**	**17.9**	**45.9**	**23.0**	**40.7**	**17.9**	**37.7**	**21.2**	**53.6**
∑ PUFA	**23.1**	**47.1**	**29.3**	**50.1**	**25.4**	**54.4**	**28.3**	**48.5**	**26.3**	**44.6**	**27.5**	**60.2**
n–3/*n*–6	**2.2**	**3.6**	**2.3**	**5.0**	**2.4**	**5.2**	**4.3**	**5.2**	**2.1**	**5.5**	**3.4**	**8.1**

Table 3 (Continued)

	Th. alalunga		*Cep. rubescens*		*Tr. lyra*		*Tr. lucerna*		*A. cuculus*		*Le. boscii*	
fatty acid	TG	PL	TG	PL	TG	PL	TG	PL	TG	PL	TG	PL
C14:0	3.3	0.3	1.8	2.3	2.6	1.4	2.9	0.3	2.8	1.1	4.6	0.7
C15:0	1.3	0.4	1.2	1.6	1.3	0.7	1.4	0.3	1.4	0.6	2.4	0.4
C16:0	25.2	16.0	18.1	17.6	20.0	15.2	20.7	14.6	13.5	15.9	20.8	16.7
C17:0	0.8	0.7	1.4	1.0	0.8	0.7	1.7	0.4	0.3	0.7	1.7	1.0
C18:0	5.9	11.7	8.5	9.7	8.2	10.7	7.8	12.5	7.8	12.0	7.9	13.4
C20.0	0.2	0.3	0.0	0.0	0.6	0.6	0.0	0.0	0.6	0.6	0.0	0.0
C24:0	0.3	1.2	1. 9	1.2	1.2	1.9	1.3	1.9	2.2	2.2	1.8	1.7
∑ saturated	**37.0**	**30.6**	**32.9**	**33.4**	**34.7**	**31.2**	**35.8**	**30.0**	**28.6**	**33.1**	**39.2**	**33.9**
C16:1	3.5	2.3	5.9	6.2	7.1	4.4	7.8	1.7	7.8	2.9	8.7	2.6
C17:1	1.9	1.1	1.6	1.3	1.7	1.7	2.5	0.8	2.0	1.0	1.1	0.0
C18:1[b]	24.4	13.7	18.0	12.2	23.6	17.6	20.5	10.6	22.4	13.2	19.4	12.1
C20:1[c]	0.9	0.6	4.7	2.5	2.6	1.0	0.9	0.5	2.6	0.7	3.7	1.0
C22:1	1.2	0.3	0.4	0.5	0.9	0.8	1.1	0.3	0.9	0.6	0.8	0.3
∑ monoenoic	**31.9**	**30.6**	**30.6**	**22.7**	**35.9**	**25.5**	**32.8**	**13.9**	**35.7**	**18.4**	**33.7**	**16.0**
C18:2 *n*–6	1.4	1.3	3.8	6.5	2.8	2.6	2.6	1.3	2.5	1.7	2.0	1.0
C20:2 *n*–6	0.5	0.2	1.5	0.0	1.0	0.7	0.1	0.0	0.5	0.0	0.0	0.0
C20:3 *n*–6	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	1.5	0.9	0.0	0.0
C20:4 *n*–6	1.0	1.2	1.7	1.6	1.7	1.8	1.3	1.8	1.6	1.5	1.7	1.9
C22:4 *n*–6	0.0	0.1	1.7	0.0	0.0	0.7	0.0	0.8	0.6	1.2	1.2	1.0
C220:5 *n*–6	0.1	0.1	1.0	0.8	0.8	1.4	0.9	0.6	0.6	1.1	1.2	0.8
∑ PUFA *n*–6	**3.0**	**2.9**	**9.7**	**8.9**	**6.4**	**7.2**	**5.0**	**4.5**	**7.3**	**6.4**	**6.1**	**4.7**
C18:3 *n*–3	0.4	0.3	1.8	1.5	1.5	0.6	1.5	0.0	0.7	1.3	1.1	0.4
C18:4 *n*–3	0.2	0.2	0.2	0.0	0.5	0.0	0.0	0.3	0.5	0.7	1.7	0.6
C20:3 *n*–3	0.0	0.1	0.2	0.0	0.1	0.0	0.0	0.6	0.5	0.2	0.0	0.0
C20:4 *n*–3	0.1	0.0	2.5	0.0	2.6	0.0	4.9	0.6	0.0	2.1	4.8	1.2
C20:5 *n*–3	4.6	7.5	9.6	10.5	9.4	8.8	9.0	16.5	11.8	10.4	4.5	10.8
C22:3 *n*–3	0.2	0.2	0.2	0.1	0.3	0.0	0.0	0.0	0.9	0.2	0.4	0.1
C22:5 *n*–3	0.6	1.3	2.0	1.4	0.1	1.2	0.0	1.8	1.7	2.3	0.0	2.0
C22:6 *n*–3	22.0	38.9	10.3	21.5	8.5	25.5	11.0	31.8	12.3	24.9	8.5	30.3
∑ PUFA *n*–3	**28.1**	**48.5**	**26.8**	**35.0**	**23.0**	**36.1**	**26.4**	**51.6**	**28.4**	**42.1**	**21.0**	**45.4**
∑ PUFA	**31.1**	**51.4**	**36.5**	**43.9**	**29.4**	**43.3**	**31.4**	**56.1**	**35.7**	**48.5**	**27.1**	**50.1**
n–3/*n*–6	**9.4**	**16.7**	**2.8**	**3.4**	**3.6**	**5.0**	**5.3**	**11.5**	**3.9**	**6.6**	**3.4**	**9.7**

[a] FID area percents were corrected to wt % according to total weight. Data are the means of five experiments performed in triplicate. Standard deviations were below 10%. 0.0 are values below 0.09. [b] Cis9-C18:1 + Cis11-C18:1. [c] Cis11-C20:1 + Cis13-C20:1

Statistical Analysis. Three independent analyses were done on five samples for each species for all antioxidant concentration and fatty acid content determinations. Statistical analyses were performed by the Mann−Whitney *U* test.

RESULTS AND DISCUSSION

Lipid Content and Fatty Acids Composition. The total muscle tissue lipid contents of the different species of Mediterranean fish and shellfish and the percentages of phospholipid (PL) and triglyceride (TG) fractions are given in **Table 2**. The fatty acid contents in the PL and TG fractions as well as the proportion of the saturated, monounsaturated, and polyunsaturated fatty acids are given in **Table 3**.

The muscle tissue of the species studied contained 1.23−5.83% fat. All species of fish and shellfish studied are characterized by high levels of *n*−3 fatty acids, lower levels of *n*−9 fatty acids, minimum levels of *n*−6 fatty acid, and an *n*−3/*n*−6 ratio of more than 1. The main fatty acids of TG and PL of muscle and edible tissue were docosohexaenoic acid (C22:6 *n*−3), eicosapentaenoic acid (C20:5 *n*−3), palmitic acid (C16:0), and oleic acid (C18:1). These four fatty acids represent 53.3−68.0% of TG and 59.6−78.8% of PL fractions.

Shellfish include both mollusks and crustaceans. Mollusks are similar in their fatty acid content of the PL and TG fractions: polyunsaturated fatty acids were the highest (TG, 51.9−61.5%; PL, 55.1−68.5%), followed by saturated (TG, 18.9−23.0%; PL, 16.9−22.8%) and monounsaturated fatty acids (TG, 17.9−29.2%; PL, 14.6−22.1%). The total *n*−3 fatty acids content (TG, 43.0−57.1%; PL, 47.7−57.9%) was found to be higher than the total *n*−6 fatty acids content (TG, 4.4−10.5%; PL, 7.4−10.6%). The most distinctive PUFAs of all five studied species of mollusks (*Mytilus edulis*, *Ostrea edulis*, *Tapes decussatus*, *Buccinulum cornea*, and *Phyllonotus trunculus*) were found to be DHA (C22:6 *n*−3) and EPA (C22:5 *n*−3), which ranged between 19.3 and 21.8%, and 14.4 and 28.9% in the TL fraction and between 24.0 and 34.0%, and 15.4 and 25.2% in the PL fraction, respectively; eicosatrienoic acid (C20:3 *n*−6) was also found, and this fatty acid was reported in mollusks, echinoderms, and bivalves (*19*, *20*). In *Squilla mantis*, a crustacean species, the amount of *n*−3 fatty acids was slighly lower as a consequence of the high concentration of saturated fatty acids in the TG fraction (41.3%), mainly due to palmitic (C16:0, 22.2%) and stearic acids (C18:0, 7.1%).

The lipid profile of cephalopods was clearly distinct from those of the rest of the species studied since it was rich in phospholipides; TGs have been reported as minor components of the flesh of cephalopods (*21*), and this agrees with what we report here. All three species of cephalopods studied (*Sepia officinalis*, *Loligo vulgaris*, and *Octopus vulgaris*) contained high proportions of C16:0 and C18:0. Oleic acid (C18:1 *n*−9) ranged from 12.1 to 19.1% in the TG fraction and from 8.1 to 9.2% in the PL fraction; C22:6 *n*−3 and C20:5 *n*−3 were found in proportions that ranged between 10.0 and 20.1%, and 9.6 and 14.3% in the TL fraction and between 24.5 and 35.9%, and 7.7 and 21.2% in the PL fraction, respectively. In the literature, it is also reported that DHA and EPA are the most characteristic acids for cephalopods, ranging between 20 and 26% and 8.3 and 17.3% of total fatty acids (*22*). Arachidonic

Table 4. Lipophilic Antioxidants Concentrations in Muscle Tissue of Different Mediterranean Marine Species of Fish and Shellfish[a]

species	CoQ_9H_2 (μg/g)	CoQ_9 (μg/g)	$CoQ_{10}H_2$ (μg/g)	CoQ_{10} (μg/g)	Vit E (μg/g)4r
Squilla mantis			2.49 ± 0.46	0.32 ± 0.12	11.70 ± 2.17
Mytilus edulis			8.37 ± 0.88	1.15 ± 0.23	8.52 ± 0.97
Ostrea edulis			4.01 ± 0.27	0.30 ± 0.08	5.77 ± 0.86
Tapes decussatus			6.07 ± 0.52	0.50 ± 0.09	10.40 ± 1.04
Buccinulum cornea			2.11 ± 0.43	0.21 ± 0.08	9.60 ± 1.08
Phyllonotus trunculus			2.22 ± 0.41	0.38 ± 0.07	7.80 ± 0.86
Sepia officinalis			7.18 ± 0.86	1.06 ± 0.23	9.61 ± 1.95
Loligo vulgaris	6.35 ± 0.21	1.90 ± 0.12	0.27 ± 0.05	0.10 ± 0.04	8.35 ± 1.13
Octopus vulgaris			2.94 ± 0.44	0.58 ± 0.17	7.42 ± 1.46
Sardina pilchardus			4.10 ± 0.80	0.98 ± 0.33	5.80 ± 0.66
Merluccius merluccius			2.50 ± 0.31	0.40 ± 0.15	6.40 ± 0.55
Serranus cabrilla	2.86 ± 0.61	0.62 ± 0.15			6.98 ± 1.03
Dicentrarchus labrax	5.08 ± 0.55	2.50 ± 0.44			6.48 ± 0.85
Sphyraena sphyraena			4.38 ± 0.76	1.01 ± 0.30	9.68 ± 1.45
Trachurus trachurus			2.78 ± 0.45	0.86 ± 0.28	5.41 ± 0.40
Centracanthus cirrus			3.96 ± 0.66	0.61 ± 0.19	6.44 ± 1.05
Trachinus draco	5.17 ± 0.41	1.47 ± 0.17	0.18 ± 0.04	0.08 ± 0.03	6.84 ± 0.90
Mullus barbatus			1.95 ± 0.30	0.49 ± 0.15	7.47 ± 1.60
Mullus surmuletus			2.28 ± 0.31	0.33 ± 0.12	7.94 ± 1.50
Pagellus erythrinus	0.18 ± 0.04	0.10 ± 0.03	2.83 ± 0.12	0.27 ± 0.06	7.09 ± 1.25
Boops boops			2.99 ± 0.63	0.74 ± 0.27	8.65 ± 1.76
Diplodus annularis			2.91 ± 0.45	0.51 ± 0.21	17.90 ± 2.40
Lithognatus marmyrus			4.02 ± 0.40	0.87 ± 0.41	10.99 ± 1.35
Scomber scombrus			3.64 ± 0.52	0.63 ± 0.13	14.24 ± 2.16
Thunnus alalunga			5.11 ± 0.93	1.13 ± 0.18	5.06 ± 0.74
Cepola rubescens			1.86 ± 0.31	0.54 ± 0.15	5.61 ± 0.90
Trigla lyra	2.49 ± 0.47	0.40 ± 0.12			10.3 ± 0.91
Trigla lucerna	2.36 ± 0.84	0.36 ± 0.15	0.28 ± 0.06	0.07 ± 0.04	7.65 ± 1.45
Aspitrigla cuculus	3.34 ± 0.56	0.77 ± 0.23			4.84 ± 0.52
Lepidorhombus boscii			1.57 ± 0.31	0.35 ± 0.15	14.71 ± 2.11

[a] Each result represents the mean ± SD of five experiments performed in triplicate.

Table 5. Hydrophilic and Enzymatic Antioxidants Concentrations in Muscle Tissue of Different Mediterranean Marine Species of Fish and Shellfish[a]

species	Vit C (μg/g)	GSH (μg/g)	GSSG (μg/g)	Cu–Zn SOD (U/mg proteins)	GPx (U/mg proteins)
Squilla mantis	3.6 ± 0.7	52.0 ± 6.3	4.2 ± 1.5	5.1 ± 1.3	0.40 ± 0.08
Mytilus edulis	4.9 ± 0.9	47.2 ± 4.1	3.3 ± 0.8	5.5 ± 0.9	0.43 ± 0.06
Ostrea edulis	3.3 ± 0.4	35.1 ± 3.4	2.4 ± 0.3	4.1 ± 0.8	0.32 ± 0.08
Tapes decussatus	2.8 ± 0.5	42.7 ± 4.3	2.7 ± 0.4	5.7 ± 1.0	0.27 ± 0.06
Buccinulum cornea	3.0 ± 0.4	31.6 ± 3.5	3.5 ± 0.6	3.8 ± 0.8	0.40 ± 0.08
Phyllonotus trunculus	3.5 ± 0.6	29.5 ± 3.7	2.9 ± 0.4	4.3 ± 0.7	0.38 ± 0.09
Sepia officinalis	2.2 ± 0.7	40.5 ± 4.6	2.1 ± 0.9	1.9 ± 0.5	0.16 ± 0.04
Loligo vulgaris	2.4 ± 0.4	38.5 ± 3.9	4.2 ± 1.4	3.8 ± 0.8	0.21 ± 0.05
Octopus vulgaris	2.7 ± 0.8	44.6 ± 5.2	3.2 ± 0.9	3.8 ± 0.8	0.17 ± 0.07
Sardina pilchardus	2.3 ± 0.5	32.0 ± 6.6	2.9 ± 0.6	9.7 ± 1.2	0.26 ± 0.05
Merluccius merluccius	2.9 ± 0.6	28.1 ± 3.6	2.8 ± 1.2	5.8 ± 1.4	0.23 ± 0.06
Serranus cabrilla	2.8 ± 0.7	22.1 ± 3.7	1.9 ± 0.4	5.8 ± 1.2	0.23 ± 0.09
Dicentrarchus labrax	6.5 ± 0.8	28.4 ± 4.3	3.1 ± 0.7	4.6 ± 0.8	0. 53 ± 0.06
Sphyraena sphyraena	4.1 ± 0.7	14.9 ± 5.6	0.6 ± 0.2	6.3 ± 1.4	0.29 ± 0.10
Trachurus trachurus	2.5 ± 0.6	38.9 ± 4.7	3.8 ± 1.2	4.6 ± 1.1	0.64 ± 0.11
Centracanthus cirrus	2.8 ± 0.7	30.0 ± 6.4	2.9 ± 0.7	4.8 ± 0.8	0.34 ± 0.06
Trachinus draco	4.6 ± 0.8	24.7 ± 3.9	2.5 ± 1.0	3.7 ± 0.4	0.33 ± 0.04
Mullus barbatus	11.4 ± 2.0	29.5 ± 6.4	2.0 ± 0.4	4.6 ± 0.6	0.35 ± 0.05
Mullus surmuletus	11.8 ± 1.7	35.0 ± 7.2	2.4 ± 0.6	4.9 ± 1.0	0.36 ± 0.04
Pagellus erythrinus	16.0 ± 1.4	30.8 ± 3.6	2.3 ± 0.8	3.5 ± 0.4	0.30 ± 0.05
Boops boops	2.7 ± 0.2	40.4 ± 3.9	3.1 ± 0.7	3.0 ± 0.6	0.18 ± 0.05
Diplodus annularis	2.5 ± 0.6	57.1 ± 6.6	5.1 ± 1.4	3.2 ± 0.7	0.19 ± 0.06
Lithognatus marmyrus	2.3 ± 0.4	34.4 ± 1.8	2.6 ± 1.2	4.1 ± 0.9	0.21 ± 0.05
Scomber scombrus	6.1 ± 1.1	34.2 ± 2.6	3.3 ± 1.6	5.7 ± 0.8	0.26 ± 0.07
Thunnus alalunga	12.1 ± 0.9	41.6 ± 3.8	2.8 ± 0.5	4.7 ± 0.8	0.20 ± 0.08
Cepola rubescens	3.0 ± 0.8	34.8 ± 3.9	2.6 ± 0.9	3.6 ± 1.0	0.20 ± 0.07
Trigla lyra	14.1 ± 2.8	28.5 ± 5.2	4.7 ± 1.3	4.2 ± 1.0	0.26 ± 0.07
Trigla lucerna	20.1 ± 4.9	18.8 ± 4.2	2.9 ± 1.1	3.9 ± 0.6	0.24 ± 0.06
Aspitrigla cuculus	2.5 ± 0.5	23.8 ± 6.4	2.6 ± 0.7	3.7 ± 1.1	0.29 ± 0.08
Lepidorhombus boscii	2.5 ± 0.4	18.6 ± 3.1	2.6 ± 0.7	2.8 ± 0.9	0.40 ± 0.04

[a] Each result represents the mean ± SD of five experiments performed in triplicate.

acid (C20:4 $n-6$) was found in proportions that ranged from 1.1 to 1.9%. Culkin and Morris (*24*) also reported arachidonic acid to represent 1−2% of the total fatty acids in cephalopod mollusks of the North Atlantic, while Gibson (*23*) found arachidonic acid to represent 9.8% of the total fatty acids in an octopus from southern Australia. These observed differences

can be explained by the variation of fatty acid composition of the diet, as a consequence of the continuous recycling of fatty acids though different food webs in the sea.

Among teleosts, the content of C22:6 *n*−3 and C20:5 *n*−3 varies from species to species, and aside from a few exceptions, these fatty acids are more abundant in the phospholipid component. **Table 3** illustrates several points of interess concerning marine fish lipids and fatty acids. The first is that PLs had high a proportion of C22:6 *n*−3, less C20:5 *n*−3, very little C22:1, and reduced C20:4 *n*−6. In contrast, the TGs had high proportions of C18:1, C20:1, and C22:1 and a more balanced ratio of C22:6 *n*−3 and C20:5 *n*−3. The second is that the PL fraction displayed high PUFA levels (34.4−60.2%) and moderate monounsaturated fatty acids concentration (9.1−28.2%), whereas the TG fraction was very rich in monounsaturated fatty acid (26.4−42.8%) and showed a PUFA content much lower than that of the PL fraction (21.7−39.4%). *Merluccius merluccius*, a fish with low fat content, also shows low C22:6 *n*−3 and C20:5 *n*−3 content. An important nutritional finding is that some species have over 30% phospholipid DHA content, including bluefishes *Sardina pilchardus*, *Scomber scombrus*, *Thunnus alalunga*, *Sphyraena sphyraena*, *Boops boops*, and *Trigla lyra* and fish with white flesh such as *Dicentrarchus labrax.*

Lipophilic Antioxidants. Lipophilic antioxidant levels are given in **Table 4**. Coenzyme Q is a lipid-soluble compound found in plants and animals in two redox forms and with varying length of the isoprenoid tail. Besides its activities in the electron-transport chain, coenzyme Q (in reduced form) has also been implicated as the only endogenously synthesizized lipid-soluble antioxidant protecting cellular membranes and plasma lipoproteins from free radical damage (*24*). Furthermore, it is able to sustain efficiently the chain-breaking antioxidant capacity of Vit E by regenerating it from tocopheroxyl radical (*6*). A derangement of these reductive mechanisms, due to an overproduction of pro-oxidant reactive species, coupled to a reduced CoQ_{10} biosynthesis, represents an important fingerprint of oxidative stress. Oxidative stress and antioxidant deficiency are linked in a reciprocal manner and can potentially produce a toxic cellular environment capable of attacking a variety of biomolecules as well as inhibiting energy production.

Dietary intake of CoQ_n may have beneficial effects on human health, but only a few papers have focused on the CoQ_n contents in foods during the past two decades. The species under study are characterized by two forms of ubiquinones: in 4 species the CoQ_9H_2−CoQ_9 is present, in 22 species the $CoQ_{10}H_2$−CoQ_{10} is present, and in another 4 species both forms are present. In this latter group, the amount of $CoQ_{10}H_2$−CoQ_{10} is lower than the CoQ_9H_2−CoQ_9 level, except in *Pagellus erythrinus*. Independently of the two forms, the CoQ_nH_2−CoQ_n content varies from species to species. In all fresh species studied, the ratio of reduced CoQ_nH_2 is high relative to the content of the oxidized form, CoQ_n, and these parameters could be useful as an index of fish freshness.

Higher levels of Vit E (D-*RRR*-α-tocopherol) were found in *Diplodus annularis*, *Lepidorhombus boscii*, and *Scomber scombrus* (17.90, 14.71, and 14.24 μg/g, respectively). This means that consumption of 100−200 g/day of the above fish is sufficient to meet the daily nutritional needs of healthy persons (Recommended Dietary Allowances) for Vit E, which are 3−4 mg/day in the U.K. and 8−10 mg/day in the U.S.A. (*25*).

β-Carotene and retinol have not been detected in the muscle tissue by our analytical methods, while remarkable amounts of retinyl palmitate, a physiological source of vitamin A, were found in the liver of all fish (data not shown).

Hydrophilic and Enzymatic Antioxidants. The glutathione system and the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), which detoxify superoxide anion (O_2^-) and H_2O_2, act a primary defense as endogenous physiological antioxidants. GSH and GSSG, ascorbic acid, SOD, and GPx levels are summarized in **Table 5**. GSH serves in a multitude of critical cellular defensive functions, including free radical scavenging, detoxification of electrophiles, and maintenance of thiol−disulfide status and signal transduction (*26*). In the different species, the GSH values ranged between 14.9 and 57.1 μg/g. The selenium-containing enzyme, glutathione peroxidase (GPx), reduces a number of peroxides, including fatty acid hydroperoxides and hydrogen peroxide, to the corresponding alcohols while oxidizing 2 mol of glutathione. GPx has been found in the muscle tissue of a number of different fish species (*26*, *27*); in our study the GPx values ranged between 0.16 and 0.40 U/mg of protein. In all fresh fish species studied, the ratio GSH/GSSG is high and could be useful as a quality parameter.

Ascorbate is a water-soluble free radical scavenger, and reduced ascorbate may be regenerated from the free radical, semidehydroascorbate, by the enzyme semidehydroascorbate reductase using NADH as the electron donor (*28*). The highest levels of ascorbic acid were found in *Trigla lucerna* (20.1 μg/g), *Pagellus erythrinus* (16.0 μg/g), *Trigla lyra* (14.1μg/g), *Thunnus alalunga* (12.1 μg/g), *Mullus surmuletus* (11.8 μg/g), and *Mullus barbatus* (11.4 μg/g).

The role of SOD is to catalyze the dismutation of the superoxide ion (O_2^-) to hydrogen peroxide and molecular oxygen during oxidative energy processes. The reaction diminishes the destructive oxidative processes in cells. In the different species, the Cu,Zn−SOD values ranged between 1.9 and 9.7 U/mg of protein. Catalase activity was undetectable by our analytical methods, indicating that in fish the activity of this enzyme is significantly lower than that reported in mammals and birds (*29*).

CONCLUSIONS

As in the Mammalia, Vit E (D-*RRR*-α-tocopherol) and reduced and oxidized forms of ubiquinones (CoQ_nH_2/CoQ_n) are the main lipophilic antioxidants in the muscle tissue of all the fish and shellfish species under study. And, as in the Mammalia, independently on the class and order, some fish and shellfish species display $CoQ_{10}H_2$/CoQ_{10}, others CoQ_9H_2/CoQ_9, and others both the coenzymes. Carotenes (α, β, γ, δ) and retinol (vitamin A) have not been detected in the muscle by our methods. Fairly good levels of enzymatic and hydrophilic nonenzymatic antioxidants share in the antioxidant pool of each marine species (Cu,Zn−SOD, 1.9−9.7 U/mg of protein; GPx, 0.18−0.40 U/mg of protein; GSH, 14.9−52.6 μg/g; GSSG, 0.6−4.2 μg/g; Vit C, 2.4−20.1 μg/g). All together, they represent a powerful defense system against the oxidation of a large variety of biomolecules, particularly *n*−3 polyunsaturated fatty acids.

Several past presentations have reviewed human epidemiological studies suggesting the benefits of consuming relatively large levels of fish oil and especially *n*−3 fatty acids. High levels of docosohexaenoic acid (C22:6 *n*−3) and eicosapentaenoic acid (C22:5 n−3) were found in the phospholipid and triglyceride fractions of all Mediterranean fish and shellfish species, the total *n*−3 fatty acid contents were found to be higher than the total *n*−6 fatty acid contents, confirming their great importance as a significant dietary source of *n*−3 polyunsaturated fatty acids.

The fish and shellfish species studied are among the most common found in the market for human consumption.

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