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Proteins, fatty acids and nutritional value in the muscle of the fish species *Mora moro* (Risso, 1810)

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Deep-water fish are becoming an interesting object of studies and research due to the development of deep fishery activities. This paper analyses the chemical composition and nutritional value of the fish species *Mora moro* (Risso, 1810) inhabiting deep Mediterranean waters. The fatty acid profile and the principal water-soluble proteins present in the white muscle of this fish species have also been determined. The major fatty acids were 22 : 6n-3, 16 : 0, 18 : 1n-9, 20 : 4n-6 and 20 : 5n-3. The polyunsaturated fatty acid (PUFA) content was higher than that of saturated (SFA) and monounsaturated fatty acids, but the ratio PUFA/SFA was lower than the value reported in other studies. Both the atherogenic index and thrombogenic index were very low. Water-soluble proteins were characterised by monodimensional native PAGE and 2-D SDS-gel electrophoresis. Protein patterns showed the presence of parvalbumins and of the principal myofibrillar proteins. Therefore, the deep-water fish *M. moro* could represent an interesting target for deep-sea fishery and commercial exploitation.

Keywords: Deep fishery / Fatty acids / *Mora moro* / Nutritional value / Proteins

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1 Introduction

Mediterranean deep fishery is carried out at 300–700 m depth as a rule, and it is mostly targeted to deep shrimps such as *Aristeus antennatus* and *Aristaemorpha foliacea*. However, in recent years some fishery activities have been carried out in deeper Mediterranean waters (below 800 m depths) in order to discover potential new resources.

With regard to bony fish species, the family Moridae is one of the most represented at the aforementioned depths as has been previously reported [1, 2]. According to Cohen *et al.* [3], the family Moridae is widely distributed in all the oceans and includes 18 genera and 87–89 putative species. Six species have been reported in the Mediterranean Sea [4]. Most of them live on slope bottoms deeper than 200 m.

The common mora (also called Morid cod) is a benthopelagic species mostly from the slope oceanic and Mediterranean areas [3]. The body colour is generally grey; the main diagnostic features of the species are the presence of a chin barbel, two dorsal fins (soft rays) and a relatively large eye [3].

The common mora – *Mora moro* (Risso, 1810) – can be considered as one of the most interesting fish species for potential fishery exploitation, according to the relative abundance in the catches from the deep Mediterranean bottoms [1, 2] and body sizes (up to 50 cm total length) in the Mediterranean [4]. Moreover, the species has been exploited by deep-sea commercial fisheries in other geographical areas (mostly France–Atlantic, Ireland and New Zealand) since 1995, and a total world production of 1250 metric tons was reported for 2001 [5]. The total species production targeting the human consumption is actually low when compared with other gadiform fish as the cod (*Gadus morhua*; 950 000 metric tons) and the European hake (*Merluccius merluccius*; 70 000 metric tons) [5]. The common mora, as along with most of the deep-water fish species, has been poorly investigated in terms of its nutritional qualities [6, 7].

In the present paper, we have evaluated some characteristics of the *M. moro*: chemical composition, energy content, fatty acid and protein composition in the muscle, as this is the most important component from the nutritional and commercial point of view.

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Muscle proteins were identified and characterised by native PAGE and 2-DE, whereas the fatty acid profile was assessed by gas chromatographic analysis.

The high content of polyunsaturated fatty acids (PUFA), the corresponding atherogenic index (AI) and thrombogenic index (TI) [8], and the quality of proteins, containing all essential amino acids, all indicate that the deep-water fish *M. moro* has a high nutritional value. Results shown in this paper can be useful for assessing the exploitation of the resource, considering that deep environments are considered fragile ecosystems and need a precautionary approach [9].

2 Materials and methods

2.1 Fish samples

Morid cod (*M. moro*) samples came from catches of experimental trawl hauls carried out on mesobathyal bottoms (800 – 1200 m depths) in the Southern Adriatic Sea (Mediterranean Sea). Fishes were stored at −20°C until analysis, which was carried out on the edible flesh (the dorsal muscle) of *M. moro*. Analyses were performed on two different aliquots of the same fish. For each aliquot, the analyses were carried out in triplicate and the results were presented as mean of three different values ± SD.

2.2 Chemical composition and energy content

For the analysis, fish samples were thawed at room temperature for 1 h, blotted with a sheet of paper, gutted and filleted. Measurements of the moisture, protein, fat, carbohydrate and ash contents were carried out according to the procedures reported in the AOAC method [10]. The energy content was calculated as: proteins, 4.27 kcal · g^{-1} wet weight; carbohydrates, 4.11 kcal · g^{-1} wet weight and lipids, 9.02 kcal – · g^{-1} wet weight (1 kcal = 4.184 kJ).

2.3 Determination of fatty acid composition

Fatty acid extraction was carried out according to the protocol described by Folch *et al.* [11] with some minor modifications. Methyl ester preparation was carried out according to the procedure reported in the AOAC method [12]. Concentrated samples were kept in sealed capillary tubes and stored at −20°C until the gas chromatographic analysis.

Gas chromatographic analysis was carried out using a Varian model 3400 chromatograph (Varian, Palo Alto, CA, USA) equipped with a flame ionisation detector (FID) and a column injection system. The carrier and make-up gases were high-purity helium at flow rates of 1.3 and 30 mL · min^{-1}, respectively. High-purity hydrogen (30 mL · min^{-1}) and compressed air (300 mL · min^{-1}) were supplied to the FID. A fused-silica capillary column (60 m × 0.25 mm id) was used for the analysis (J&W Scientific, Folsom, CA, USA). The injection and detector temperatures were programmed at 250 and 300°C, respectively, while column temperature was programmed as follows: 90°C for 2 min, from 90 to 180°C at 10°C · min^{-1}, from 180 to 240°C at 5°C · min^{-1} after 10 min hold at 180°C and at 240°C for 16 min. Samples (1 µL) were injected in Spittles mode (spittles time: 30 s). Gas chromatographic analysis was carried out in triplicate on two different extracts from the same sample. Fatty acid esters were identified by means of the retention time of chromatographic standards (Sigma) analysed under the same experimental conditions. Peak areas were determined using the Varian software.

AI and TI were determined according to the Ulbricht and Southgate equations as described in Ref. [8]. In these indices, different weights are attributed to different categories of fatty acids – saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and PUFA of both the n-3 and n-6 categories – in relation to their different contributions to the prevention or promotion of coronary heart diseases:

$$AI = (\text{Lauric} + 4*\text{Myristic} + \text{Palmitic}) \times (\text{PUFA¤-3} + \text{PUFA n-6} + \text{Oleic} + \text{other MUFA})^{-1}$$

$$IT = (\text{Myristic} + \text{Palmitic} + \text{Stearic}) \times (0.5*\text{Oleic} + 0.5*\text{Other MUFA} + 0.5*\text{PUFA n-6} + 3*\text{PUFA n-3})$$

2.4 Extraction of water-soluble protein from white muscle

The edible flesh of *M. moro* was recovered and pooled in duplicate. Muscle portions were cut into small pieces and homogenised at a ratio of 1 : 5 w/v with 10 mM sodium phosphate buffer pH 7.0 by means of an Ultra-Turrax homogeniser (9500 rpm for 5 min, with interruptions every 30 s). The homogenates obtained were centrifuged at 12 000 × *g* for 15 min at 4°C and the supernatants were filtered on Whatman n.3 filters. Protein content was determined according to Bradford [13] using the BioRad reagent in the microassay mode and BSA as a standard.

2.5 Native PAGE

Native PAGE was performed according to Schägger and von Jagow [14], using a 4% spacer gel and 12% running gel. Proteins were solubilised with a medium containing

12% glycerol, 0.01% bromophenol blue, 50 mM Tris-HCl, pH 6.8. Gel was stained with CBB and scanned using an ImageMaster DTS (Pharmacia Biotech) scanner.

2.6 2-DE

Aliquots of protein extract (900 µg protein) were dried in speed vac and resuspended with 450 µL of a rehydratation solution containing 7 M urea, 2 M thiourea, 2% CHAPS, 20 mM DTT and 0.5% IPG buffer plus a trace of bromophenol blue and then added to a 24 cm IPG DryStrip pH 4–7 linear (Amersham Biosciences) [15, 16].

IEF was run on an IPGphor unit (Amersham Biosciences) for 51 000 Vh. After IEF, the IPG-strip equilibration steps were carried out in 1% DTT containing solution (6 M urea, 30% glycerol, 2% SDS, 50 mM Tris-HCl, pH 8.8) and then in the same solution with 4% iodoacetamide plus a trace of bromophenol blue [17].

The IPG strip was laid on a homemade 15% homogeneous polyacrylamide slab gel on the Ettan DALT II system (Amersham Biosciences). The run was performed for 14 h at 10°C, 16 W. The gel was stained with CBB and scanned using an ImageMaster DTS (Pharmacia Biotech) scanner. Image analysis was performed using ImageMaster 2-D Elite V. 2002.01 software (Amersham Biosciences).

2.7 Protein identification by MALDI-TOF analysis

Spots of interest were excised from the stained native PAGE and 2-D gels and subjected to in-gel trypsin digestion. Briefly, excised spots were washed, reduced, S-alkylated and digested with trypsin (Promega, Madison, WI, USA; modified trypsin) as described elsewhere [18]. The resulting peptide mixtures were analysed using MALDI-TOF MS.

For MALDI-TOF MS analysis, 2 µL of each peptide mixtures was mixed (1 : 1) with matrix solution (10 mg · mL^{-1} CHCA in 0.5% TFA, 50% ACN). Subsequently, 0.3 µL of this matrix–peptides mixture was applied in duplicate on a sample slide tray and allowed to air dry. Mass spectra were acquired in reflectron mode using an Ettan MALDI-TOF Pro mass spectrometer (Amersham Biosciences). Peptide masses (M + H) in the range 800–3000 Da were measured. Spectra were calibrated using two internal standard peptides (^{ile7}AngIII, M + H 897.531, monoisotopic, and hACTH 18–39, M + H 2465.191, monoisotopic).

Mass finger-printing database searching was carried out using the Mascot software available online (http://www.matrixscience.com).

3 Results

The edible part of deep-water fish *M. moro* was analysed as described in Section 2. The proximate chemical composition *per* 100 g wet weight was: 77.73 ± 0.36% moisture, 19.16 ± 0.17% protein, 1.06 ± 0.11% lipid and 2.36 ± 0.17% ash. Carbohydrate content was not detectable. As a result, the corresponding energy value was 361.0 ± 7.5 kJ.

As determined by gas chromatographic analysis, about half of the fatty acid content of the edible muscle of *M. moro* is represented by PUFA. Sixty-five percent of them is represented by docosahexanoic acid (22 : 6n-3), 14.5% by arachidonic acid (20 : 4n-6) and 12.0% by eicosapentaenoic acid (20 : 5n-3), respectively. About one-third of the fatty acids are represented by SFA, in particular by palmitic acid, whereas MUFA represent only one-sixth of the total fraction, with the oleic acid being the most frequent among them. Results are shown in Table 1.

Table 1. Relative percentages of fatty acid in the white muscle of deep fish *M. moro*, as determined by gas chromatographic analysis

Fatty acids	Composition (% of total fatty acids)	SD
C 14 : 0	0.65	0.02
C 16 : 0	25.74	0.33
C 16 : 1n-7	1.94	0.03
C 17 : 0	0.40	0.04
C 17 : 1	0.43	0.02
C 18 : 0	6.88	0.17
C 18 : 1n-9	11.35	0.33
C 18 : 1n-7	2.03	0.09
C 18 : 2n-6	0.51	0.00
C 18 : 3n-3	0.20	0.00
C 20 : 1n-7	0.87	0.06
C 20 : 2n-6	0.17	0.01
C 20 : 4n-6	7.12	0.15
C 20 : 5n-3	5.83	0.15
C 22 : 1n-11	0.17	0.02
C 24 : 0	0.67	0.02
C 21 : 5n-3	1.45	0.02
C 22 : 5n-3	1.74	0.03
C 22 : 6n-3	31.85	0.50
SFA	34.34	0.62
MUFA	16.79	0.56
PUFA	48.87	0.82
PUFA/SFA	1.42	
n-3/n-6	5.26	
AI	0.44	0.04
TI	0.15	0.02

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; AI: atherogenic index; TI: thrombogenic index. Values are mean with SD of two pooled samples. Analysis was carried out for each sample in triplicate.

Both the calculated AI (0.44 ± 0.04) and TI (0.15 ± 0.02), reported in the same table, were very low.

Analysis of water-soluble protein extracts by native gel electrophoresis revealed the presence of seven bands (Fig. 1). Six of them were identified by MS, as follows: band 2: creatine kinase; band 3: myosin heavy chain (MHC), bands 4 – 6: skeletal α-actin; band 5: β-parvalbumin and band 7: myosin light chain 3 (MLC3).

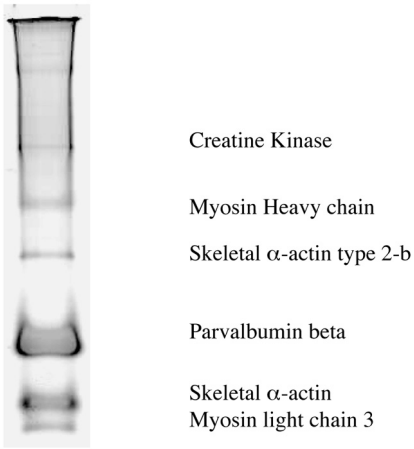

Figure 1. Native gel electrophoresis (12% acryl.) of the water-soluble protein fraction present in the white muscle of common mora (*M. moro*). Gel was stained with CBB G-250. Bands were excised from the gel, digested with trypsin and analysed by MALDI-TOF MS for protein identification.

Figure 2 shows the 2-DE analysis of water-soluble proteins extracted from white muscle. Protein identification was achieved by MALDI-TOF MS. Forty of the 71 spots detected in 2-DE map were identified. Most of them did correspond to the principal myofibrillar proteins. In particular: five spots as α-actin, four spots as myosin light chain 1 (MLC1), two spots as myosin light chain 2 (MLC2), two spots as MLC3, three spots as MHC, three spots as Troponin T and one as tropomyosin. Three spots were represented by β-parvalbumin isoforms. Other spots were related to cell biology and in particular to energy metabolism, such MEK as enolase and triosephosphate isomerase, or to stress response. The list of identified proteins is shown in Table 2.

4 Discussion

Deep-water fishes are becoming an interesting target of studies and research as they are taken into consideration for their possible commercial exploitation. Since they live in fragile ecosystems, it is important to characterise these fishes in terms of chemical composition and nutritional value [7] and to assess their suitability for fishery [9]. The aim of the present paper was to study the biochemical composition and the nutritional value of the morid cod *M. moro*, a medium – large demersal species relatively abundant on the upper slope bottoms (1000 – 1200 m) of the Mediterranean [1, 2]. The analysis was carried out on the white muscle only.

When compared with the data reported in previous studies on *M. moro* [7, 19], our results indicate slightly higher levels of protein and lipid and lower water content. These differences can be ascribed to the different conditions of storage, but it cannot be excluded that the different locality (Mediterranean, North Sea or North Atlantic) and season of catching may play a role.

In our study, we observed that the predominant SFA was palmitic acid (16 : 0), whereas the most common MUFA was oleic acid (18 : 1n-9), and the predominat PUFA were docosahexaenoic acid, arachidonic acid and eicosapentaenoic acid, 22 : 6n-3, 20 : 4n-6 and 20 : 5n-3, respectively. These results are in good accord with those recently reported by Okland *et al.* [19] the values corresponding to arachidonic acid and eicosapentaenoic acid are inverted and but the ratio PUFA/SFA is different, being 1.42 instead of 3.6. This is due to the higher percent of SFA determined in our sample. As shown in Tab. 1, the n-3/n-6 ratio in muscle was 5.3 instead of 7.4 reported by Okland *et al.* The percent of n-3 of total fatty acid content (41.1) was close to the value reported by Okland *et al.* (55.8) [19].

As shown in numerous studies, coronary heart diseases are largely influenced by diet in correlation with the characteristic lipid profile [20]. In order to measure the propensity of this fish to influence the incidence of coronary heart disease, we have calculated both the AI and the TI. These indices might be used as a tool to compare the health quality of the lipid fraction of different foods. The AI value obtained was lower than those for other animal foods such as lamb (1.00), beef (0.72), pork (0.69), chicken (0.50) and rabbit (0.82) [21] but similar to those reported for finfish foods [12].

The low fat content, the predominance of n-3 PUFA, such as docosahexanoic acid, and the low values of the AI and TI make this fish appropriate for reducing blood plasma lipids and thus the risk of coronary heart disease [20].

Moreover, the proteomic approach to the composition of the dorsal muscle of *M. moro* has revealed the presence of proteins of high quality containing all the essential amino acids as in similar foods of animal origin. Most of the proteins identified in our study – enzymes of energy metabolism and proteins of contractile apparatus – have been recently found also in zebrafish skeletal muscle [22]. The effect of hypoxic exposure on the pattern of protein expression, shown in this latter study, might be of interest for a

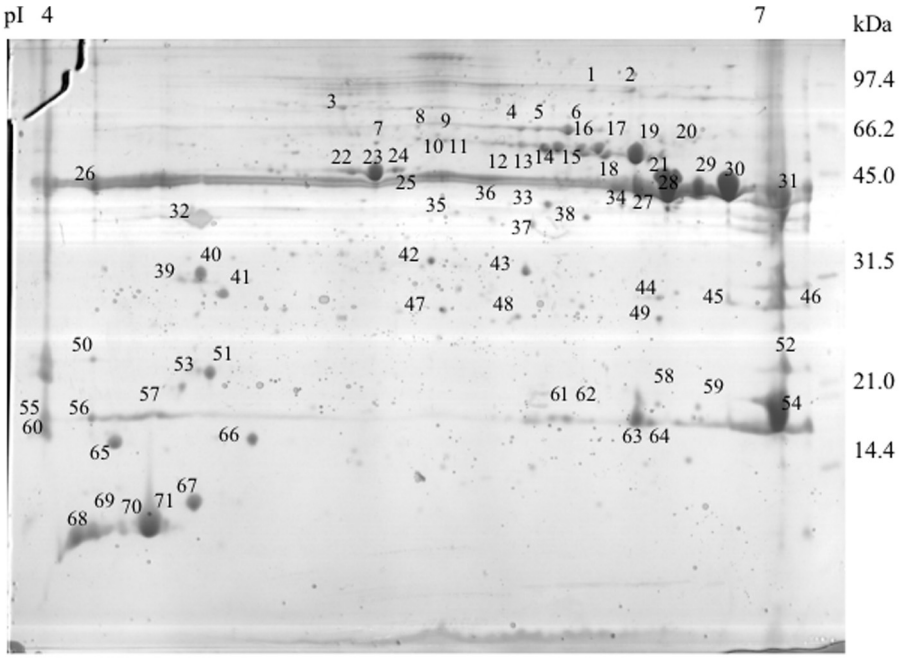

Figure 2. 2-DE (1-D: pH 4–7 linear; 2-D: 15% acryl.) of the water-soluble protein fraction present in the white muscle of common mora (*M. moro*). Gel was stained with CBB G-250. Spot numbers were excised from the gel, digested with trypsin and analysed by MALDI-TOF MS for protein identification.

Table 2. Water-soluble proteins present in white muscle of deep fish *M. moro* identified by MALDI-TOF MS

Spot number	p*I*	MW kDa	Homology	Species	Accession number	Score	Coverage %
3	5.19	71.52	Heat shock protein 70	*Ictalurus punctatus*	gi/1346318	45	12
4	5.14	110.25	MHC	*Cyprinus carpio*	gi/806515	65	14
5	5.14	110.25	MHC	*C. carpio*	gi/806515	65	14
6	5.14	110.25	MHC	*C. carpio*	gi/806515	65	14
14	5.33	40.133	α1 enolase-1	*Salmo trutta*	gi/11999265	50	18
15	5.33	40.133	α1 enolase-1	*S. trutta*	gi/11999265	50	18
17	5.33	40.133	α1 enolase-1	*S. trutta*	gi/11999265	50	18
19	5.73	51.28	Keratin type IIS	*Acipenser baerii*	gi/32452103	30	14
22	5.23	42.28	Actin, α skeletal muscle	*Carassius auratus*	gi/762889	95	31
23	5.25	42.19	Skeletal α actin type 2-b	*Coryphaenoides armatus*	gi/30268605	61	37
24	5.28	42.18	Skeletal α actin	*Sparus aurata*	gi/6653228	98	27
25	5.28	42.18	Skeletal α actin	*S. aurata*	gi/6653228	62	20
27	6.44	42.98	Muscle type creatine kinase	*Oreochromis mossambicus*	gi/21694043	68	22
28	6.44	42.98	Muscle type creatine kinase	*O. mossambicus*	gi/21694043	68	22
29	6.44	42.88	Creatine kinase muscle isoform 2	*Chaenocephalus aceratus*	gi/31322099	65	18
30	6.44	42.88	Creatine kinase muscle isoform 2	*C. aceratus*	gi/31322099	65	18
31	8.09	36.50	Fructose-bisphosphate aldolase A	*Oryzias latipes*	gi/46849371	45	13
32	4.75	32.27	Tropomyosin	*Theragra chalcogramha*	gi/27127288	55	15
33	6.29	44.47	cMEK1	*C. carpio*	gi/17974311	46	15
39	4.70	21.17	Fast skeletal MLC1a	*C. carpio*	gi/27127288	55	15
40	4.77	21.63	MLC1	*Engraulis japonicus*	gi/7678758	45	18
41	4.69	20.69	MLC1	*Cipselurus agoo*	gi/7678740	40	19
42	5.09	27.75	Mixed lineage leukemia-like protein	*Psenopsis anomala*	gi/38426594	45	35
45	7.60	27.76	Triosephosphate isomerase b	*Xiphophorus maculatus*	gi/15149252	68	29
46	7.60	26.76	Triosephosphate isomerase b	*X. maculatus*	gi/15149252	59	29
47	5.28	42.18	Skeletal α actin	*S. aurata*	gi/6653228	48	21
49	5.73	29.80	Fast skeletal MHC	*G. morhua*	gi/20563006	39	13
51	4.72	19.09	Myosin regulatory light chain 2	*Salmo salar*	gi/30141484	69	55
52	7.08	27.70	Unnamed protein product	*Tetraodon nigroviridis*	gi/47213334	53	22
53	4.72	18.99	Myosin regulatory light chain 2	*C. carpio*	gi/6729202	58	29
54	9.68	26.63	Fast myotomal muscle troponin T	*S. salar*	gi/3264817	39	15
55	4.87	13.93	Immunoglobulin heavy chain	*G. morhua*	gi/7981155	30	25
60	4.34	17.67	MLC3	*Decapterus maruadsi*	gi/16117357	82	29
63	9.68	26.63	Fast myotomal muscle troponin T	*S. salar*	gi/3264817	39	15
64	9.68	26.63	Fast myotomal muscle troponin T	*S. salar*	gi/3264817	39	15
65	4.34	17.67	MLC3	*D. maruadsi*	gi/16117357	86	29
66	4.70	21.34	MLC1	*Scomber japonicus*	gi/16117365	30	19
68	4.56	11.61	β-Parvalbumin	*G. morhua*	gi/14531014	40	42
69	4.56	11.61	β-Parvalbumin	*G. morhua*	gi/14531014	40	42
70	4.56	11.61	β-Parvalbumin	*G. morhua*	gi/14531014	40	42

better understanding of *M. moro* metabolism and gene expression.

Altogether, the high-nutritional value of *M. moro*, its low fat content, the good quality of its proteins, and its low impact on atherogenic and thrombogenic diseases makes this fish very suitable for commercial exploitation. Moreover, previous studies [7] indicated the good acceptability of the fish through sensorial analysis, the common mora fish fillet being more accepted than the cod (*G. morhua*). However, the species exploitation have to be carefully managed according to the assessment of the overall impact of deep-water fishing on the ecosystems involved [23].

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