

Identification by proteome analysis of muscle proteins in sea bream (*Sparus aurata*)

Roberta Schiavone · Loredana Zilli · Carlo Storelli ·
Sebastiano Vilella

Received: 11 September 2007 / Revised: 31 January 2008 / Accepted: 6 March 2008 / Published online: 19 March 2008
© Springer-Verlag 2008

Abstract The present paper reported postmortem changes in muscle proteins on sea bream (*Sparus aurata*), during ice storage. The postmortem evolution of protein patterns in farmed sea bream was monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and two-dimension electrophoresis (2DE). Matrix associated laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) was used for identification of proteins. Results of the bio-informatics analysis demonstrate that: (1) the normalized volume of skeletal alpha-actin and tropomyosin is not affected by the time of storage; (2) the normalized volumes of myosin light chain 3 and of major histocompatibility complex (MHC) class II beta1 increase; (3) the normalized volumes of Sec 13-like and parvalbumin significantly decrease.

Keywords Sea bream · Fish muscle · Proteins · 2D-electrophoresis · MALDI-TOF

Introduction

Food safety and food quality are important issues nowadays. Therefore, it is important to keep the quality of fish at a high level in each link of the complex fishery chain in order to be able to guarantee the consumer a fresh and high quality end product. The quality of fish as nourishment is determined by a variety of factors including freshness [1, 2].

The postmortem loss of freshness, which in turn determines loss of quality, is the result of complex processes (microbiological, physiological, chemical, and biochemical) that start with the bacterial proliferation following the production of nutrients by endogenous (hydrolytic) enzymes. Among these hydrolytic reactions, proteolysis determines changes in the texture and tenderization of fish muscle [3, 4]. These changes are species dependent and reflect seasonal variations [5–8]. Although different methods have been established [1] for the evaluation of fish freshness, the identification of muscle proteins that changes during postmortem storage in ice could be potentially useful to set up, in the future, quick methods to determine fish freshness.

In particular, up to date no studies have been performed on *Sparus aurata* concerning protein changes in fish muscle occurring during *postmortem* storage in ice. This information can be obtained by the proteome analysis technique [9]. Proteome analysis is a promising technique for providing new information on biochemical changes occurring in fish during postmortem tenderization. In fish research, proteome analysis has been applied to different fields [9–13]. With respect to protein deterioration processes occurring in fish muscle during ice storage, proteome analysis has been reported only for sea bass, cod, and salmon. Proteome analysis of muscle protein patterns in sea bass (*Dicentrarchus labrax*) revealed that three protein spots could be used (after protein identification) as markers of freshness, since they progressively decreased during ice storage [14]. Kjærsgård and Jessen [2] reported that in cod (*Gadus morhua*), 11 protein spots (with an MW ranging between 13 and 35 kDa) significantly changed during ice storage (nine increased while two decreased). Interestingly the two protein spots that decreased showed a molecular size (≈ 15 kDa) similar to those of the three sarcoplasmic

R. Schiavone (✉) · L. Zilli · C. Storelli · S. Vilella
Laboratorio di Fisiologia Generale e Comparata,
Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali,
Università del Salento, Via Prov. Monteroni, 73100 Lecce, Italy
e-mail: roberta.schiavone@unile.it

proteins of sea bass (≈ 16 kDa) that significantly changed during ice storage. Also, in salmon fillets, stored on ice, protein degradation depends upon time and is mainly due to the endogenous enzymes [15].

The matrix-associated laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is the most commonly used technique for identification of proteins after two-dimension electrophoresis (2DE).

In the present study, the postmortem evolution of protein patterns of sea bream muscle was monitored by 2DE and the image analysis applied to select protein spots used for the MALDI-TOF analysis.

Materials and methods

Animal and tissue preparation

Six sea bream (*Sparus aurata*) (250–450 g) during January 2006 were obtained from a local fish farm (MARIBRIN s.r.l. Brindisi, Italy) and brought back alive and fasting to the laboratory. The fish were slaughtered in water and ice (40 min), as the normal procedure in Italian fish farms, and placed in insulated keeping boxes containing flakes of ice and stored at 4–6 °C [16, 17]. Sampling was performed as follows: with a biopsy needle, a piece of muscle was taken from the fish, the skin was removed, and the central core of the muscle was used for protein extraction, thereby ensuring that the muscle proteins used for proteome analysis had not been exposed to ice or air. Muscle samples were extracted from the loin muscle, under the dorsal fin and above the lateral line. The fish were kept in ice for 6 days, and muscle samples were extracted after 0, 24, 48, 72, 96 and 144 h, referring to the time of the first sample excision as time zero. The fish were in rigor between 24 and 48 h. Samples were frozen in dry ice and kept at -80 °C before extraction.

Protein extraction, isoelectric focusing (IEF) and SDS-PAGE

Extraction buffer for total protein extracts contained 8 M urea, 0.5 mM PMSF, 4% (w/v) CHAPS, and 65 mM DTE in 40 mM Tris-base. A sample of 0.3 g directly taken from the dorsal white fish muscle was homogenized in 3 mL of extraction buffer in a Potter RGL 100 (Heidolph, Kelheim, Germany) for 1 min. The extracts were centrifuged at $9,200\times g$ for 10 min and the clear supernatant was collected. Protein content of this final extract was measured by the Quick Start Bradford Protein assay (Bio-Rad) using bovine gamma-globulin as standard. IEF was performed on immobilized pH-gradients (IPG, pH 3–10, 13 cm) with IPGphor (Amersham Biosciences). A total of 60 μ g of

protein was used for analytical runs and 800 μ g proteins for preparative runs to a total volume of 250 μ l rehydrating buffer [8 M urea, 2% (w/v) CHAPS, 18.6 mM dithiothreitol (DTT) and 1% IPG- buffer pH 3–10 (Amersham Biosciences). The strips were rehydrated for 12 h. Focusing was performed with 50 μ A per strip for 1 h to 500 V, 1 h to 1,000 V and 2 h to 8,000 V at 20 °C. After IEF, the strips were equilibrated first step with 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 50 mM Tris, pH 6.8, and 2% (w/v) dithiothreitol for 15 min and in the second step with 2.5% (w/v) iodoacetamide instead of dithiothreitol for another 15 min. As tracking dye, few grains of bromophenol blue were added. Separation of the second dimension was performed in 12.5% SDS/polyacrylamide gels (14 $\times$ 16 cm) using the Hofer SE 600 Ruby System (Amersham Biosciences). The running conditions were 15 mA/gel for 15 min and 30 mA/gel for 5 h. Once the bromophenol blue had reached the anode, the gels were fixed and stained by a standard silver staining protocol (Amersham Biosciences). In preparative gels, protein spots were visualized by Coomassie R-250 staining (Roti-Blue, Roth, Karlsruhe, Germany) following the manufacturer's protocol.

Acquisition and analysis of 2D gels

The stained 2D gels were scanned on ImageMaster Gel Scanner (Amersham Biosciences). The image analysis was done using the Imagemaster 2D Elite software 3.1 (Amersham Biosciences). Protein spots were detected using automated routines from the software combined with manual editing to remove artifacts. For each fish, one gel from muscle samples extracted after 0 h was selected as the basis for the construction of a reference gel against which the remaining gels were matched using standard routines from within the software. Each spot within the reference gel was assigned a spot number that was used in the subsequent description to refer to individual spots.

Tryptic in gel digestion of 2DE-resolved proteins

Spots of interest were excised from a Coomassie blue stained 2D gels. The gel pieces were put in 0.5 ml Eppendorf tubes and destained overnight with a solution of 5 mM ammonium bicarbonate/50% acetonitrile. After removing the supernatant, the gel pieces were dehydrated by acetonitrile, swelled by rehydration in 5 mM ammonium bicarbonate and shrank again by addition of acetonitrile [18]. After removing the liquid phase, the gel pieces were dried in speedvac and the proteins were digested overnight at 37 °C with trypsin (Promega, Madison, WI, USA; modified trypsin) and the resulting peptide mixtures were analyzed by MALDI-TOF mass spectrometry.

MALDI-TOF mass spectrometry analysis

For MALDI-TOF mass spectrometry analysis 2 μ l of each peptide mixture was mixed with 2 μ l of α -cyano-4-hydroxy-cinnamic acid solution in 50% v/v acetonitrile/0.5% v/v trifluoroacetic acid (TFA). Subsequently, 0.4 μ l of this matrix–peptides mixture was applied to a target disk and allowed to air dry. Spectra were acquired using an Ettan MALDI-TOF Pro mass spectrometer (Amersham Biosciences). 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 fingerprinting database searching was carried out using Profound (<http://prowl.rockefeller.edu/prowl-cgi/profound.exe>), Mascot (<http://www.matrixscience.com>), and MS-FIT (<http://prospector.ucsf.edu>), online available softwares. The comparison of the search in different databases is a valuable check of the protein identification by informatics analysis [19]. The search parameters were as follows: allowed one missed cleavage, oxidation of methionine and carbamidomethylation of cysteine.

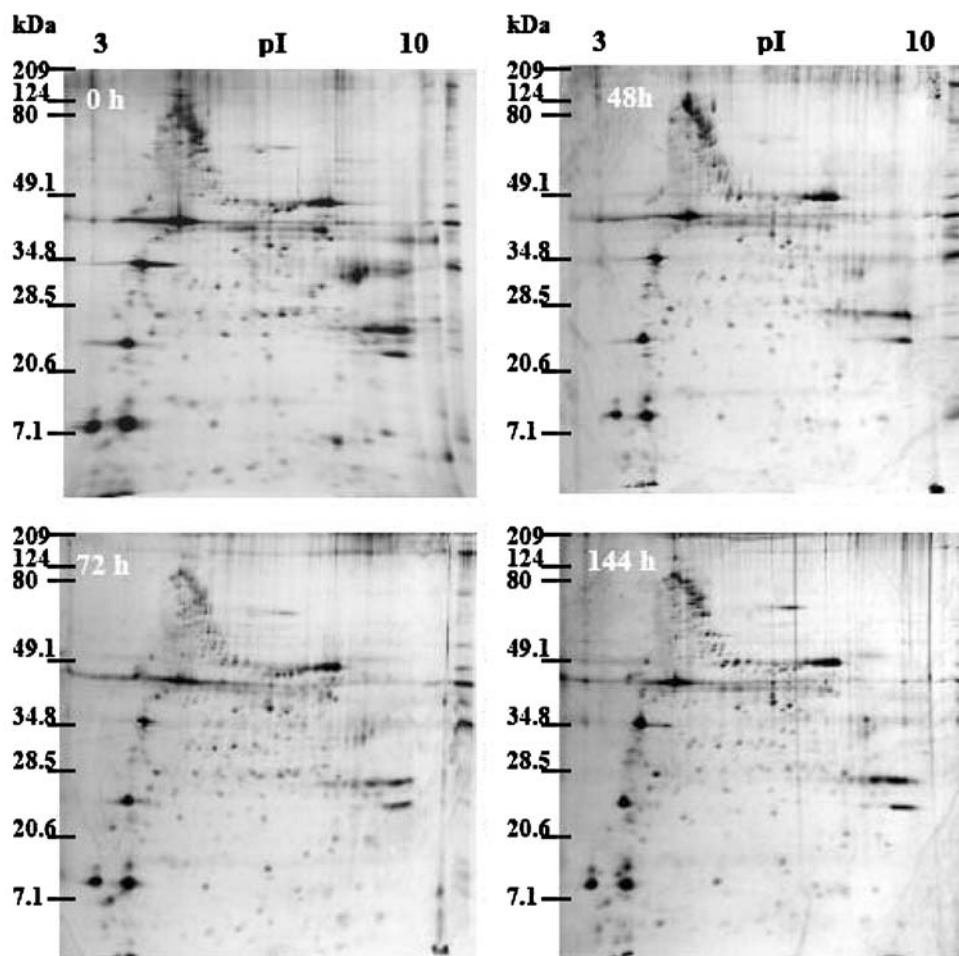
Statistical analysis

The amount of protein present in a spot was taken as the area of the spot multiplied by the density and referred to as the volume. Following removal of background, the spot volumes were normalized to the total protein detected for each gel by dividing the individual spot volume by the sum of all spot volumes and multiplying by 100. The normalized spot volume is referred to as abundance. Normalized volume of each spot considered in this study was evaluated as mean $\pm$ standard deviation of spot normalized volumes measured in all gels produced at the same storage time. Comparison of proteins at different times of ice storage was assessed using the Mann–Whitney test and relationships were considered statistically significant when $p < 0.05$.

Results and discussion

The two-dimensional experiment, after staining, revealed 800–1000 protein spots (Fig. 1). However, we focused our study on the partial proteome with the optimal resolution

Fig. 1 2-D PAGE of total proteins from sea bream muscle extracted from fish kept on ice 0, 48, 72 and 144 h



(Fig. 2), since for spots corresponding to proteins at higher molecular weight (>60 kDa) the resulting resolution was scarce. Of course, all the big cytoskeletal proteins of the fish muscle, that are known to be degraded during the postmortem processes [7–9, 20, 21] were not present in the partial proteome analyzed in this study, although some spots that showed a time-dependent increasing level during postmortem storage in ice could represent their degradation products (Fig. 3).

The protein profiles of the samples at different time scales showed some differences first on visual inspection, and subsequently by using image analysis software. Among these spots, we chosen ten spots (named 2, 5, 6, 7, 9, 10, 11, 12, 13, 14 and highlighted in Fig. 2) for the further analysis. The criteria used to select spots were: (a) spots that were present in all gels at time 0; (b) isolated spot; (c) significant difference in protein quantities (normalized spot volume) between different time of storage on ice; (d) a high-normalized

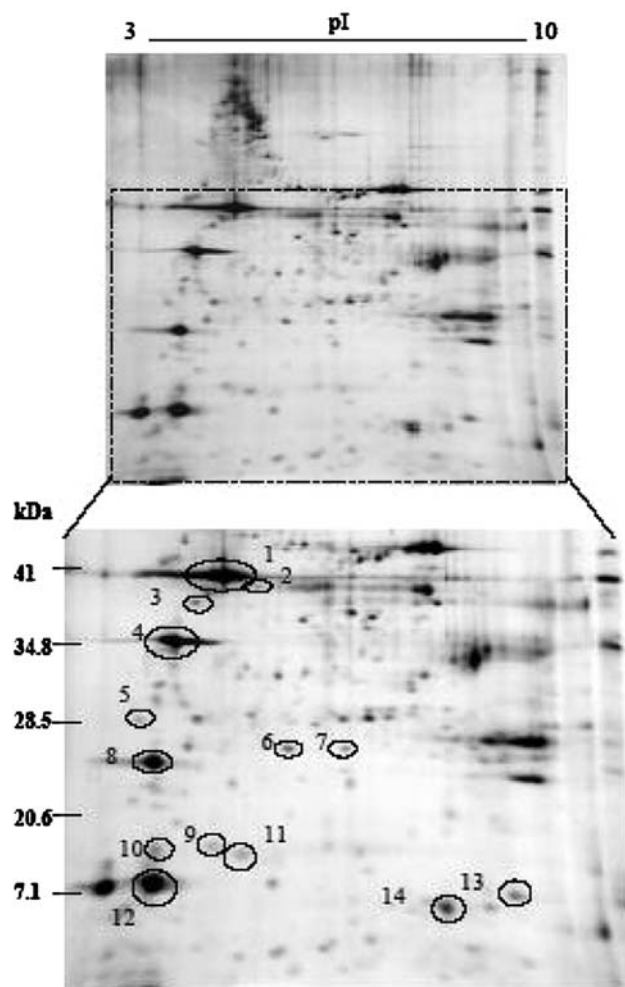


Fig. 2 2-D PAGE of sea bream muscle proteins. The dotted line square specifies the area in the gels used for further proteome analysis. The spots marked were analyzed with MALDI-TOF for protein identification

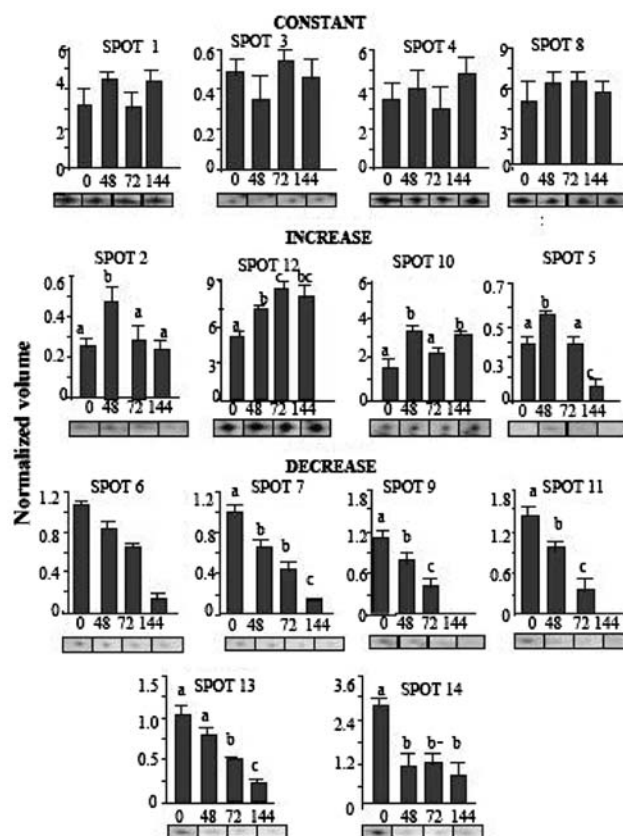


Fig. 3 Expression profiles of 14 postmortem proteins. The proteins are highlighted in Fig. 2. Along the x-axis each of the 14 histograms shows the relative abundance at the four postmortem times, 0, 48, 72 and 144 h. The y-axis corresponds to the intensity of spots expressed as normalized spot volume. Spot intensity is expressed as mean $\pm$ SD of normalized spot volume measured in 18 different gels (six different fish and three replicates for each). Different letters in the same graph indicate that mean values are significantly different by Mann–Whitney test ($p < 0.05$)

volume (to simplify the detection of the spot in the preparative gel and to obtain a sufficient amount of each protein). We also selected for further analysis, as control, four spots (named 1, 3, 4, 8 and highlighted in Fig. 2) with a high-normalized volume that showed no changes during storage. It must be underlined that no significant differences were observed, under our experimental conditions, in the spot levels between 24 and 48 h and between 72 and 96 h (data not shown). For this reason data concerning the storage times 24 and 96 h were omitted from the figures. Among the selected spots two (named 2, 5) showed a significant ($p < 0.05$) increase followed by a decrease, while the spots 10 and 12 showed a significant ($p < 0.05$) increase followed by stagnation. Similar changes have been observed also for cod muscle stored on ice [2] and could be due to the shift in metabolic processes [22], or to synergistic model of action between different proteases [23] or both [2]. The remaining spots (6, 7, 9, 11, 13, 14) showed a consistent ($p < 0.05$) decrease. In particular, protein spots 9 and 11 completely

disappeared at 144 h (Fig. 3). Proteome analysis of muscle protein patterns in salmon, sea bass and cod revealed that also in these species, due to the action of endogenous enzymes, some spots progressively decreased during ice storage [2, 14, 15]. All the changes that we observed (increase and decrease) could be the result of the changes in the metabolism that occur in the fish muscle after death. As previously reported [2], the lack of oxygen determined a shift in the metabolism that in turn resulted in first a decrease of ATP concentration followed by ATP synthesis from creatine phosphate, ADP and glycogen. These mean that all the enzymes involved must be upregulated [24]. Therefore, the proteome changes in postmortem sea bream muscle could reflect either synthesis of new proteins, protein degradation, changes in solubility, or a combination of these factors. Of course, all these processes are greatly

affected by the antemortem conditions of fish and/or storage conditions.

For protein identification, peptide fragment masses were used to search different databases for protein homologies using three search programs. Protein homologies were obtained for nine spots. These proteins were identified from protein sequences already described in other teleost species and amphibians. The identified proteins are listed in Tables 1, 2 and 3 together with origins of the matched proteins sequences, the theoretical molecular weight of the proteins, the databases used in the search and the sequence coverage of the matched peptides in the database search.

Among the four protein spots that remained unchanged (Table 1) during ice storage, only one (spot number 8) was not identified. Spots 1 and 3 showed homology with a skeletal alpha actin of *Sparus aurata*, while spot number 4 with

Table 1 Results from peptide mass fingerprinting of protein spots that did not change during postmortem storage on ice

Reference spot	WMe (kDa) PIe	MASCOT Homology	Pro-found Homology	MS-FIT
1	43 5.3	Skeletal alpha actin	Skeletal alpha actin	Actin muscle
		(<i>Sparus aurata</i>)	(<i>Sparus aurata</i>)	(HALRO)
		Coverage 25%	Coverage 25%	Coverage 58%
		WMt: 42.19 kDa PIt: 5.28	WMt: 42.19 kDa PIt: 5.3	WMt: 42 kDa PIt: 5.2
3	40 5.2	Skeletal alpha actin	Skeletal alpha actin	Actin. alpha 1, skeletal muscle
		(<i>Sparus aurata</i>)	(<i>Sparus aurata</i>)	(<i>Danio rerio</i>)
		Coverage 25%	Coverage 20%	Coverage 64%
		WMt: 42.19 kDa PIt: 5.28	WMt: 42.19 kDa PIt: 5.3	WMt: 41.98 kDa PIt: 5.2
4	33 4.9	Tropomyosin 3	Tropomyosin	Tropomyosin
		(<i>Danio rerio</i>)	(<i>Pennahia argentata</i>)	(<i>Pennahia argentata</i>)
		Coverage 21%	Coverage 21%	Coverage 53%
		PMt: 32.93 kDa PIt: 4.71	PMt: 32.77 kDa PIt: 4.7	PMt: 32.73 kDa PIt: 4.7

Table 2 Results from peptide mass fingerprinting of protein spots that increased during postmortem storage on ice

Reference spot	WMe (kDa) PIe	MASCOT Homology	Pro-found Homology	MS-FIT
10	10 4.8	Myosin light chain 3	Myosin light chain 3	Myosin light chain alkali
		(<i>Caranx delicatissimus</i>)	(<i>Caranx delicatissimus</i>)	(<i>Danio rerio</i>)
		Coverage 30%	Coverage 31%	Coverage 62%
		PMt: 16.64 kDa PIt: 4.37	PMt: 16.63 kDa PIt: 4.4	PMt: 16.94 kDa PIt: 4.5
12	9 4.8	MHC class II beta 1	MHC class II beta 1	–
		(<i>Salvelinus namaycush</i>)	(<i>Salvelinus namaycush</i>)	
		Coverage 32%	Coverage 32%	
		PMt: 8.1 kDa PIt: 5.58	PMt: 8.1 kDa PIt: 5.9	

Table 3 Results from peptide mass fingerprinting of protein spots that decreased during postmortem storage on ice

Reference spot	WMe (kDa) PIe	MASCOT Homology	Pro-found Homology	MS-FIT
7	22	Sec13-like protein	Sec13-like protein	–
	6.0	(<i>Danio rerio</i>)	(<i>Danio rerio</i>)	
		Coverage 28%	Coverage 12%	
		PMt: 21.27 kDa	PMt: 21.27 kDa	
9	10	Unnamed protein	Unnamed protein	–
	5.0	Coverage 48%	Coverage 48%	
		PMt: 12.4 kDa	PMt: 12.39 kDa	
		PIt: 5.74	PIt: 5.7	
11	9	Parvalbumin 1	Parvalbumin 1	Parvalbumin beta (LATCH)
	5.2	(<i>Rivulus marmoratus</i>)	(<i>Rivulus marmoratus</i>)	
		Coverage 21%	Coverage 21%	
		PMt: 11.71 kDa	PMt: 11.7 kDa	
13	9	Unnamed protein	Unnamed protein	–
	8.6	Coverage 37%	Coverage 37%	
		PMt: 9.74 kDa	PMt: 9.73 kDa	
		PIt: 9.7	PI: 10.0	

tropomyosin 3. Actins played key roles in different cellular functions, showed tissue specificity, and were highly conserved [25]. The presence of alpha skeletal actin has been demonstrated in white and red muscle of *Sparus aurata* [26] as well as in other fish species [27, 28]. The present data confirm the previous findings and demonstrate that *Sparus aurata* alpha skeletal actin was not degraded during the first 144 h of storage in ice (Fig. 3). Our results demonstrate that also the normalized volume of *Sparus aurata* muscle tropomyosin did not change during ice storage (Fig. 3). Similar to alpha skeletal actin, also the presence of tropomyosin has been demonstrated in different fish species [2, 14, 15]. Altogether the present data demonstrate that both actin and tropomyosin are relatively stable under post-mortem storage in ice and are in agreement with previously reported results [2, 14, 15].

Among the spots that showed a significant ($p < 0.05$) increase of the normalized volume (Table 2), only two (named 10 and 12) were identified by informatics analysis: spot 10 showed homology with myosin light chain 3 and spot 12 with MHC class II beta 1.

Myosin, the major protein component of striated muscle tissues, consists of two heavy chains (MHCs) and four light chains (MLCs), which combine to form a long coiled alpha-helical tail and two heads. Each head contains an actin-binding site and ATPase activity. The formation of the head structure involves the N-terminal half of two MHCs and one pair of light chains [29]. The light chains

belong to the troponin-C supergene family [30] and are classified into two groups: the alkali- and dinitrobenzoic acid (DTNB)-removable light chains [31]. Fish skeletal muscle myosins contain two alkali light chains, MLC1 and MLC3, and a regulatory light chain, MLC2 [32–38]. The increase that we observed could be due to a degradation of the myosin heavy chain (200 kDa) that might lead to an increase of myosin light chains.

Similar results have been obtained in cod [2]. On the other hand, myosin has been found to degrade in cold-water shrimp (*Penaeus borealis*), but not in warm-water shrimp (*P. japonicus* and *P. monodon*) [39].

The genes of the major histocompatibility complex encode two classes of glycoproteins that present the peptides to T-cells. MHC genes have been isolated from both bony and cartilaginous fish [40]. Our results showed that the normalized volume of spot 12 (identifies as MHC class II beta 1) increased during the storage in ice (Fig. 3). The increase (followed by stagnation) that we observed could represent an initial increase in protein translation due to a shift in metabolic processes, as also proposed by Kjærsgård and Jessen [2]. To the best of our knowledge no information are available on the changes occurring in these glycoproteins during postmortem storage in ice.

Among the spots that showed a significant ($p < 0.05$) decrease in the normalized volume (Table 3), spot 7 showed homology with Sec 13-like protein and spot 11 with parvalbumin 1.

Yeast Sec13p is a component of the yeast coat protein II (COP II) complex mediating the process of vesicle budding from endoplasmic reticulum (ER) to Golgi apparatus [41, 42]. Like yeast Sec13p, human Sec13 protein is essential for the transport of ER to Golgi and is a component of mammalian COP II complex as a coat and adapter protein [42, 43]. In addition, the subcellular localization of Sec13 has been observed on the membranes of the nuclear pore in yeast and mammalian cells [44]. To the best of our knowledge, no information is reported on the presence of Sec13p in fish.

Parvalbumins belong to a family of calcium-binding proteins (MW 10–13 kDa and acidic *pI* values) that play an important role in muscle relaxation [45, 46] and are present in all vertebrates including fish and amphibians [47]. Parvalbumins are known to be the major allergen from different fish species [48–52]. Amino acid sequences of parvalbumins from several fish species and amphibians have been determined and show significant sequence homology [53, 54]. The most conservative amino acid sequences are localized in the regions that form the two calcium-binding sites [53]. Based on the comparison of amino acid sequences, parvalbumins are divided into α - and β -phylogenetic lineages [55]. The α -parvalbumins have a *pI* of 5.0 or higher, while the β -parvalbumins contain more acidic amino acids, resulting in a *pI* value of 4.5 or lower. Members of both lineages have been identified in a number of fish species [47]. The distribution of the parvalbumins differs according to fish species, stage of development and muscle type [56]. Parvalbumins are found mostly in striated skeletal muscle with the white muscle generally containing more parvalbumin than the red muscle [57]. The time-dependent reduction of the normalized volume of spot 11 (identified as parvalbumin 1) that we observed (Fig. 3) could be due to degradation of the molecule or to the formation of parvalbumin aggregates as suggested previously [57].

In conclusion, our results suggest that similar to what happens in other fish species, also in sea bream the muscle protein pattern changed during ice storage with some spots being increased, some decreased and some unchanged. Between the identified proteins, those that showed a time-dependent decrement of the normalized spot volume (Sec13 and parvalbumin) could be used as a marker of sea bream freshness.

References

- Ólafsdóttir G, Martinsdóttir E, Oehlenschläger J, Dalgaard P, Jensen B, Undeland I, Mackie IM, Henehan G, Nielsen J, Nilsen H (1997) Trends Food Sci Technol 8:258–265
- Kjærsgård IVH, Jessen F (2003) J Agric Food Chem 51:3985–3991
- Busconi L, Folco EJ, Martone CB, Sanchez JJ (1989) J Food Biochem 13:443–451
- Delbarre Ladrat C, Cheret R, Taylor R, Verrez-Bagnis V (2006) Crit Rev Food Sci Nutr 46:409–421
- Brown WD (1986) In: Bechtel PJ (ed) Muscle as food. Academic Press, San Diego, CA
- Tsuchiya H, Kita S, Seki N (1992) Nippon Suisan Gakkaishi 58:793–798
- Papa I, Alvarez C, Verrez-Bagnis V, Fleurence J, Benyamin Y (1996) J Sci Food Agric 72:63–70
- Verrez-Bagnis V, Noel J, Sautereau C, Fleurence J (1999) J Food Sci 64:240–242
- Martinez I, Solberg C, Lauritzen K, Ofstad R (1992) Appl Theor Electrophor 2:201–206
- Martinez I, Christiansen JS, Ofstad R, Olsen RL (1991) Eur J Biochem 30:743–753
- Jessen F (1996) Refrigeration and aquaculture. In: Proceedings of the meeting of commission C2, International Institute of Refrigeration, Paris, France, pp 363–370
- Pineiro C, Barros-Velazquez J, Sotelo CG, Gallardo JM (1999) z Lebensm Unters Forsch 208:342–348
- Ataman C, Celik U, Rehbein H (2006) Eur Food Res Tech 222:99–104
- Verrez-Bagnis V, Ladrat C, Morzel M, Noel J, Fleurence J (2001) Electrophoresis 22:1539–1544
- Morzel M, Verrez-Bagnis V, Arendt EK, Fleurence J (2000) J Agric Food Chem 48:239–244
- Poli BM, Parisi G, Scappini F, Zampacavallo G (2005) Aquacult Inter 13:29–49
- Huidobro A, Mendes R, Nunes ML (2001) Eur Food Res Technol 213:267–272
- Shevchenko A, Wilm M, Vorm O, Mann M (1996) Anal Chem 68:850–858
- Perkins DN, Pappin DJ, Creasy DM, Cottrell JS (1999) Electrophoresis 20:3551–3567
- Tsuchiya H, Seki N (1991) Nippon Suisan Gakkaishi 57:1133–1139
- Papa I, Taylor RG, Astier C, Ventre F, Lebart MC, Roustan C, Ouali A, Benyamin Y (1997) J Food Sci 62:917–921
- Mackie IM (1993) Food Rev Int 9:575–610
- Jiang ST (1998) Proc Natl Sci Counc Repub China B 22:97–107
- Eskin NAM (ed) (1990) Biochemistry of foods. Academic Press, San Diego, CA
- Pollard TD, Cooper JA (1986) Ann Rev Biochem 55:987–1035
- Moutou KA, Socorro S, Power DM, Mamuris Z, Canario AV (2001) Comp Biochem Physiol B 130:13–21
- Venkatesh B, Tay BH, Elgar G, Brenner SJ (1996) Mol Biol 259:655–665
- Morita T (2000) Fish Sci 66:1150–1157
- Lowey S (1994) In: Engel AG, Franzini-Armstrong C (eds) Myology. McGraw-Hill, New York
- Periasamy M, Strehler E, Garfinkel L, Gubits R, Ruiz-Opazo N, Nadal-Ginard BJ (1984) Biol Chem 259:13595–13604
- Weeds AG, Lowey S (1971) J Mol Biol 61:701–725
- Rowlerson A, Scapolo PA, Mascarello F, Carpena E, Veggetti A (1985) J Muscle Res Cell Motil 6:601–640
- Ochiai Y, Watabe S, Hashimoto K (1988) Comp Biochem Physiol 90B:347–353
- Hirayama Y, Kanoh S, Nakaya M, Watabe S (1997) J Exp Biol 200:693–701
- Hirayama Y, Kobiyama A, Ochiai Y, Watabe S (1998) J Exp Biol 201:2815–2820
- Johnston IA, Cole NJ, Abercromby M, Vieira VLA (1998) J Exp Biol 201:623–646
- Xu Y, He J, Tian HL, Chan CH, Liao J, Yan T, Lam TJ, Gong Z (1999) DNA Cell Biol 18:85–95

38. Hill JA, Kiessling A, Devlin RH (2000) *Can J Fish Aquat Sci* 57:939–950
39. Martinez I, Friis TJ, Careche M (2001) *J Sci Food Agr* 81:1199–1208
40. Grimholt U, Getahun A, Hermesen T, Stet RJM (2000) *Dev Comp Immunol* 24:751–763
41. Pryer NK, Salama NR, Schekman R, Kaiser CA (1993) *J Cell Biol* 120:865–875
42. Lederkremer GZ, Cheng Y, Petre BM, Vogan E, Springer S, RA Schekman R, Walz T, Kirchhausen T (2001) *Proc Natl Acad Sci USA* 98:10704–10709
43. Tang BL, Peter F, Krijnse-Locker J, Low SH, Griffiths G, Hong W (1997) *Mol Cell Biol* 17:256–266
44. Siniosoglou S, Wimmer C, Rieger M, Doye V, Tekotte H, Weise C, Emig S, Segref A, Hurt EC (1996) *Cell* 84:265–275
45. Lannergren J, Elzinga G, Stienen GJ (1993) *J Physiol* 463:123–140
46. Muntener M, Kaser L, Weber J, Berchtold MW (1995) *Proc Natl Acad Sci USA* 92:6504–6508
47. Gerday CH (1982) *Mol Physiol* 2:63–87
48. Bugajska-Schretter A, Grote M, Vangelista L, Valent P, Sperr WR, Rumpold H, Pastore A, Reichelt R, Valenta R, Spitzauer S (2000) *Gut* 46:661–669
49. Van Do T, Hordvik I, Endresen C, Elsayed S (1999) *Scand J Immunol* 50:619–625
50. Shiomi K, Hayashi S, Ishikawa M, Shimakura K, Nagashima Y (1998) *Fisheries Sci* 64:300–304
51. Shiomi K, Hamada Y, Sekiguchi K, Shimakura K, Nagashima Y (1999) *Fisheries Sci* 6:943–948
52. Hamada Y, Tanaka H, Ishizaki S, Ishida M, Nagashima Y, Shiomi K (2003) *Food Chem Toxicol* 41:1149–1156
53. Swoboda I, Bugajska-Schretter A, Verdino P, Keller W, Sperr WR, Valent P, Valenta R, Spitzauer S (2002) *J Immunol* 168:4576–4584
54. Hilger C, Thill L, Grigioni F, Lehnert C, Falagiani P, Ferrara A, Romano C, Stevens W, Hentges F (2004) *Allergy* 59:653–660
55. Goodman M, Pechere JF, Haiech J, Demail J (1979) *J Mol Evol* 13:331–352
56. Focant B, Huriaux F, Vandewalle P, Castelli M, Goessens G (1992) *Fish Physiol Biochem* 10:133–143
57. Dory D, Chopin C, Aimone-Gastin I, Gueant JL, Guerin L, Sainte-Laudy J, Moneret-Vautrin DA, Fleurence J (1998) *Allergy* 53(1):42–50