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Structural and ultrastructural changes on muscle tissue of sea bass, *Dicentrarchus labrax* L., after cooking and freezing

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Abstract

Fish flesh undergoes structural changes during postmortem storage and processing, which could significantly influence flesh quality. This study is aimed to characterize the structural changes of the muscle tissue of the sea bass in the fresh raw state (3 h postmortem), and after different treatments (cooking and thawing).

Ten reared sea bass (approximate body length 30–40 cm) were used and muscle tissue processed in fresh raw state (FR), after cooking for 5 or 10 min (FC5 or FC10), after thawing (ThR), and after thawing/cooking (ThC5, ThC10). Light and electron microscopy techniques were used to describe and quantify muscle changes after the different processing methods. Additionally, morphometry was used to estimate muscle fibre size and percentage of interstitial material.

In FR samples typical early postmortem muscle tissue changes were observed: fibre to fibre detachment, detachment of myofibrils to endomysium, increase of the intermyofibrillar spaces, together with swelling of some organelle, mainly mitochondria and sarcoplasmic reticulum, and the appearance of abundant vesicles within the muscle fibres. Cooking in boiling water produced massive protein coagulation and shrinkage of muscle fibres with subsequent water loss. Thus, the connective tissue (collagen), sarcolemma and myofibrils lost their typical ultrastructural features. The interfibrillar spaces showed abundant amorphous material, which was slightly higher in specimens with smaller fibres and in muscle samples cooked for 10 min. Myofibrils were packed or distorted and abundant electron dense granular aggregates appeared at interstitial and subsarcolemmal spaces.

In ThR muscle samples the formation of ice crystals during the freezing process produced abundant clear spaces occupied by liquids at the interstitial spaces and inside the muscle fibres. The reciprocal arrangement of thick versus thin contractile filaments was altered in transversal sections, most sarcolemmas were broken and the intermyofibrillar spaces significantly increased. Cooking of thawed muscle samples caused massive protein coagulation and disintegration of myofibrils. The most significant feature in these samples was the appearance of intrafibrillar cavities (holes) within the muscle fibres, which were occupied by liquids, amorphous material and granular aggregates. Also, endomysium was often replaced by a dense "chain like"

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line of small granular aggregates. Correlation between the structural changes, as described in the present study, and textural and organoleptic characteristics would contribute to define the optimal conditions of postmortem processing of sea bass flesh.
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Keywords: Sea bass; Fish muscle; Flesh; Structure; Freezing; Cooking

1. Introduction

Axial skeletal muscle is the major tissue component of fish and responsible of their nutritional and commercial value. Commercial fish includes a broad number of wild and reared species, which differ significantly in their morphology, physiology, nutritional habits and lifestyles. The study of each particular species is important in order for a better understanding of the flesh quality in fish.

In teleosts the axial musculature is arranged into series of myotomes which have a complex three-dimensional morphology. The main muscle fibres types are grouped in separated muscle layers. The superficial red muscle is a thin layer of slow twitch non-fatigable muscle fibres which are commonly used for sustained slow swimming (Bone, 1966; Johnston et al., 1972; Mosse and Hudson, 1977). Red muscle fibres have abundant mitochondria, and high fat and glycogen content (Johnston, 1980; Shindo et al., 1986; Ayala et al., 1999). The white (deep) muscle has a fast twitch rapidly fatigable activity, appropriate for sudden rapid swimming (Bone, 1966; Mosse and Hudson, 1977; Carpenè et al., 1982). White muscle fibres are filled of myofibrils and have scarce mitochondria in a subsarcolemmal situation (Sänger et al., 1990). With the exception of some groups of fish like *Thuna* sp., whose red muscle is higher than usual, in most teleost species the white muscle commonly occupies most of the myotome thickness (Webb, 1970; Nag, 1972; Greer-Walker and Pull, 1975). Thus, white muscle samples are normally used to describe the structural properties of the flesh (Johnston, 1999; Johnston et al., 2000a).

Muscle fibre diameter has been demonstrated to be an important determinant of the textural characteristics of the flesh (Hatae et al., 1984, 1990; Hurling et al., 1996; Johnston et al., 2000b). Thus, textural comparison among different teleost species has shown a direct relationship between average muscle fibres size and firmness of the raw flesh, such that species with firmer texture had rather smaller fibres than species with softer texture (Hatae et al., 1984, 1990). One of the best parameters to characterize muscle cellularity in each particular species is muscle fibre density (number of muscle fibres per unit of area) (Johnston et al., 2000a,b,c, 2003a,b). White muscle fibre density varies with physiological stages, and shows considerable plasticity with respect to exercise, feeding and environmental factors (Johnston, 1999). In salmonids this parameter has been correlated with the texture and colour of the flesh (Johnston et al., 2000b).

The connective and adipose tissues are minor components of the axial musculature but also important for determining the chemical and organoleptic properties of the flesh (Fauconneau et al., 1995; Johnston et al., 2000b). The connective tissue content of the flesh depends on species specific factors such as swimming performance (Sato et al., 1986; Ofstad et al., 1996a). In raw flesh collagen maintains cohesiveness and a higher content of collagen has been correlated with a lower tenderness of the raw fish meat (Hatae et al., 1984; Sato et al., 1986). However, the collagen content of the flesh is lower than in mammalian meat (Sato et al., 1989) and less thermostable (Dunajski, 1979; Fauconneau et al., 1995; Buxadé, 1997), what creates a softer texture in heated flesh.

Cooking produces important changes in the muscle components (water, muscle fibres, connective and adipose tissues). In cod (*Gadus morhua*, L.) and Atlantic salmon (*Salmo salar*, L.) the structural changes induced by heating of muscle differ according to species, degree of postmortem ageing prior to cooking and lifestyle (wild or farmed) (Ofstad et al., 1996b). The structural changes induced by heating influences texture and other parameters associated to flesh quality. Thus, it has been observed that the texture of cooked flesh depends on the size of muscle fibres after cooking (Hatae et al., 1984, 1990; Hurling et al., 1996), the quantity of coagulated proteins in the

interstices, and the gel formed by collagen and lipids, which allows sliding of the fibres and myomeres (Hatae et al., 1990; Fauconneau et al., 1995).

Freezing is a usual method to preserve commercial fish since it stops chemical and microbiological degradation, and is an excellent method of preserving the organoleptic attributes of fish flesh during prolonged periods of time (Careche et al., 1999). However, some structural alterations may occur when freezing and, particularly during the subsequent storage of frozen flesh. Destruction of cellular compartmentalization, protein coagulation, myofibrillar aggregation, water loss (dehydration), decrease of water-holding capacity after thawing and changes in flavour and taste are the more usual effects of freezing (Fauconneau et al., 1995; Careche et al., 1999; García et al., 1999; Hall, 2001). These changes strongly depend on the species (García et al., 1999), the methodology of freezing (slow or rapid rates) (Love, 1968; Bello et al., 1982), the storage time and temperature (Careche et al., 1999). All these parameters notably influence flesh quality, i.e. for lean species of high commercial value such as hake (*Merluccius merluccius*, L.), the end of practical storage life is reflected as a fibrous, dry product which becomes tough and has lost important functional properties (Careche et al., 1999).

Sea bass, *Dicentrarchus labrax* L., is a teleost widely distributed in the Mediterranean and Atlantic seas. This species is intensively farmed in many Mediterranean countries (Greece, Spain, Italy, etc.), and highly appreciated because of its excellent organoleptic properties and reasonable prize. However, similarly to other farmed teleosts like the Atlantic salmon (Sheehan et al., 1996), intensive production levels plus nutritional and managing factors may lead to an associated decline in flesh quality which could produce a long term refuse effect by consumers. Therefore, a characterization of the structural, physical and chemical properties of the flesh seems to be necessary in order to define the standard of quality of the farmed sea bass. Several previous works have described the development and muscle fibre cellularity of the axial musculature of the sea bass (Scapolo et al., 1988; Veggetti et al., 1990; Ramírez Zarzosa et al., 1995; López-Albors et al., 1998). Also, some investigations have been carried out aimed to characterize the postmortem changes in the muscle tissue of this species from pre- to post-rigor state (Papa et al., 1996, 1997; Verrez-Bagnis et al., 1999, 2002; Ladrat et al., 2003). However, the effect of cooking as well as the combined effect of freezing/thawing/cooking has not been studied yet. Thus, the present work is focused to describe the structural and ultrastructural changes in the axial musculature of farmed sea bass by effect of freezing and cooking. This work may be a necessary first step for standardizing flesh quality of farmed sea bass.

2. Material and methods

Specimens were reared at the Instituto Español de Oceanografía (Centro Oceanográfico de Murcia, Puerto de Mazarrón) from a selected spawn of 740,000 eggs from spawners of Atlantic sea bass adapted to captivity. Prelarvae were maintained in darkness during the vitelline phase and subsequently exposed to continuous light. At 18 days, photoperiod was changed to 12:12 light/darkness. Larvae were initially fed with *nauplii* of *Artemia salina* and later it was replaced by *Artemia metanauplii*. Postlarvae were fed "ad libitum" with commercial granular feed (Trouw, S.A.). Specimen cultivation was performed at ambient temperature—minimum of 13 °C in January, and maximum of 26 °C in August—in tanks of 2 m^3 until a weight of 10–15 g, and subsequently in tanks of 6 m^3 until commercial size (≅ 30–40 cm). Oxygen level was measured with an oximeter (Oxiguard Mk III) and maintained over 6 p.p.m. Ten specimens were harvested after 24 h without feeding. They were randomly sampled, anaesthetized with clave oil (Guinama^R) and then killed by decapitation. Subsequently they were measured (fork length), weighed (Table 1) and delivered to the Veterinary Faculty of Murcia in sealed cages with ice pellets.

2.1. Muscle sample preparation

Fish were kept on ice within 3 h postmortem. Subsequently, axial muscle samples were obtained by removal of two pieces (0.5 cm. thickness) of the whole left half cross-section of the axial musculature, just caudally (piece I) and craneally (piece II)

Table 1
Length and weight of fish, and corresponding muscle cellularity parameters

Specimen length (cm)	Specimen weight (g)	Average muscle fibres diameter (μm) ± S.E.M	Muscle fibres density (number/mm^2)
29.5	330	76.43 ± 1.9	120.58
29	240	99.23 ± 2.7	80.8
30	260	119.72 ± 6.05	40.65
32.3	360	91.78 ± 1.88	90.83
33.5	460	86.07 ± 9.9	100.6
33.5	510	90.32 ± 2.35	110.01
34	370	80.68 ± 2.8	120.29
36.5	460	74 ± 3.5	120.64
39	760	86.9 ± 2.42	110
41	800	72 ± 3.18	150.94

to the anal opening (Fig. 1). Pieces I and II were processed in raw state or after cooking, respectively. Piece I was cut into two half (epaxial and hypaxial) at the level of the horizontal septum. The epaxial (dorsal) half was processed in fresh and the hypaxial (ventral) wrapped with kitchen foil and frozen at −20 °C for 24 h. Hypaxial samples were weighted before freezing as well as after thawing in order to calculate water loss by thawing. Piece II was also cut into two halves, both of them being cooked, immediately (epaxial) or after freezing for 24 h at −20 °C (hypaxial). The hypaxial half of pieces I and II were thawed in the fridge (4 °C) for 12–16 h. Before cooking, the epaxial and hypaxial half of piece II were weighed and put into vacuum sealed plastic bags. These bags were then immersed in boiling water for 5 or 10 min. After a rest of 20 min at ambient temperature, cooked samples were weighed again in order to calculate water loss by cooking. According to this methodology flesh samples from each fish were labelled as fresh raw (FR), fresh cooked (FC5 and FC10), thawed raw (ThR) and thawed cooked (ThC5 and ThC10).

2.2. Muscle sample processing, staining and viewing

Fresh samples were trimmed into small blocks of approximately 1 × 1 × 0.5 cm (Fig. 1). Half of the blocks were frozen in 2-methylbutane (−80 °C), snap frozen over liquid nitrogen, and then stored in a −65 °C freezer until sectioning. Sections of 8 μm thickness were obtained at −20 °C in a cryostat (Leica CM 1850), and stained with Haematoxylin/Eosin, and for myosin ATPase reaction after acid preincubation pH 4.6 (15–30 s) (method B, Mascarello et al., 1986). mATPase staining was used to reveal denaturation of contractile proteins by freezing/thawing or cooking. These slides were used for both structural and morphometric studies by light microscopy. The half of blocks left was processed for scanning and transmission electron microscopy (T.E.M.). T.E.M blocks, properly trimmed to obtain transversal sections were fixed in 2.5% glutaraldehyde in buffered 0.1 M cacodylate (pH 7.2–7.4) for 3 h, at 4 °C. Further T.E.M. processing was performed in the Servicio Universitario de Microscopía Electrónica (SUME, University of Murcia), according to standard protocol for epoxy embedding method. Ultrathin sections were obtained using a Reicher Jung (Heidelberger, Germany) ultramicrotome and then stained with aqueous saturated uranyl acetate and Reynold lead citrate and viewed in a Zeiss EM 109 and EM 10C (München, Germany) transmission electron microscopes at 80 kV. Samples for scanning electron microscopy were fixed in 2.5% glutaraldehyde, post fixed with osmium tetroxide and then dehydrated with acetone. The critical point was carried out at the SUME (University of Murcia) with acetone 100% and liquid CO_2. The samples were finally metallized with Biot-Rad Polaron Division (200 Amstrong) and viewed in a Jeol 6100 scanning microscope.

2.3. Morphometry and quantification of changes

The morphometric analysis by light microscopy was carried out by means of an image analysis system device (Kontron 100, Zeiss) connected to a light photomicroscope (Leitz Dialux 20). In all specimens ($n = 10$) and flesh samples (FR, FC5, FC10, ThR, ThC5 and ThC10) a minimum of 200 cross-sectioned white muscle fibres were measured (diameter) by outline tracing, throughout five to seven fields of 0.19 mm^2. Also, percentages of myofibrillar (contractile) and interstitial materials were calculated. Additionally, the percentages of fibre to fibre detachment (according to methodology previously used in salmon by Taylor et al., 2002), as well as the percentages of fibre fragmentation and intrafibrillar cavitation (holes), were calculated for a total

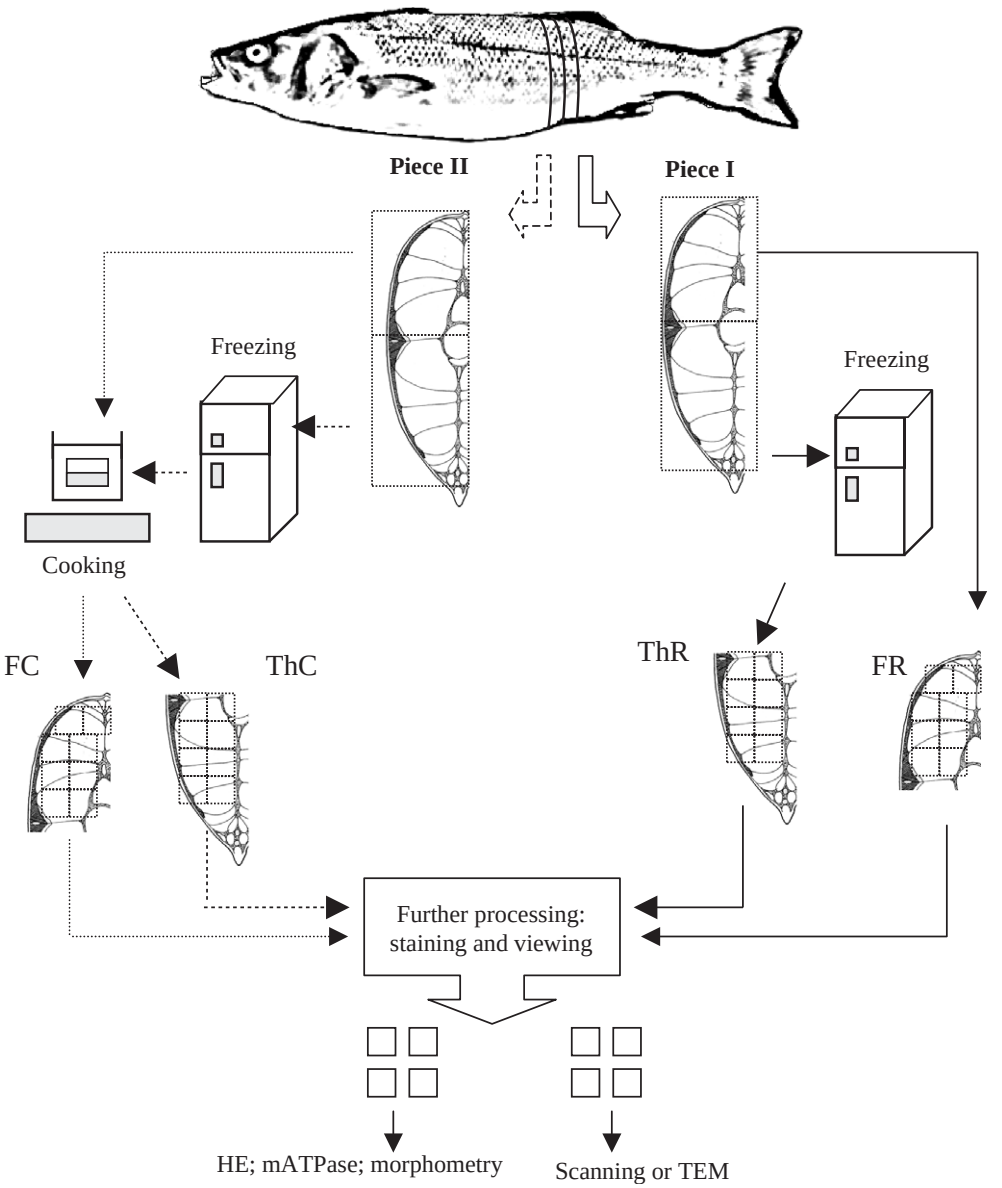

Fig. 1. Muscle sample processing. FR, FC, ThR and ThC correspond to samples processed in fresh raw, fresh cooked, thawed raw and thawed cooked states, respectively.

number of 50 fibres from each flesh sample and specimen.

Quantification of ultrastructural parameters was based on previous studies in fish and mammals meat (Ho et al., 1996; Taylor and Koohmaraie, 1998; Taylor et al., 2002) and was carried out by direct T.E.M. viewing. Thus, the percentages of myofibrils detached to endomysium by cytoskeleton (costameres) were calculated in 5 randomly selected specimens. In each specimen and muscle sample, 50–75 myofibrillar attachments to endomysium were counted in each one of 7–10 randomly selected muscle fibres. The percentages of increased inter-myofibrillar spaces were calculated in a similar

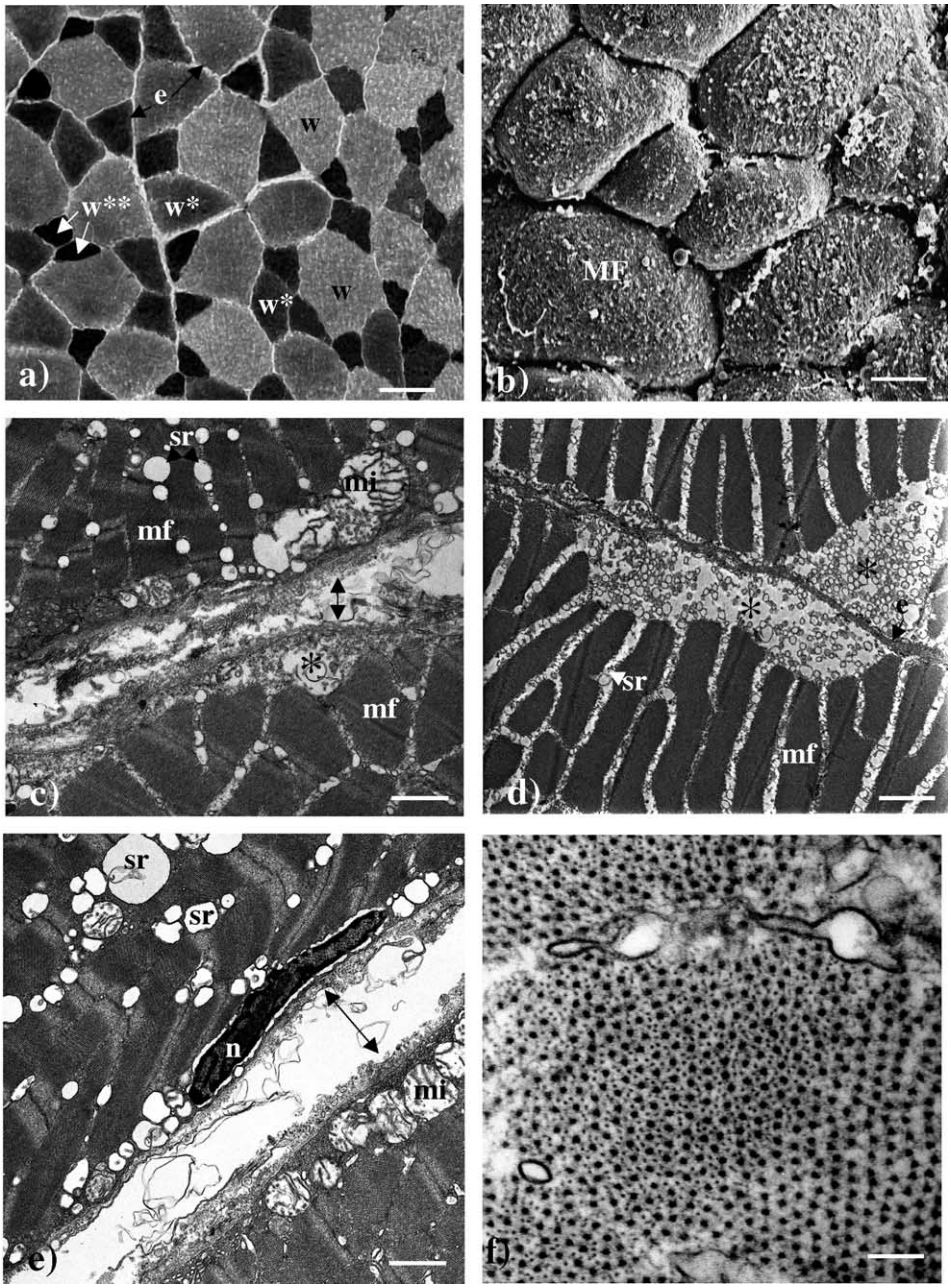

Fig. 2. Fresh raw (FR) muscle samples of sea bass 3 h postmortem. (a) mATPase staining after acid preincubation pH 4.6 (30 s). Mosaic aspect of the white muscle fibres. Bar: 50 μm (b) Transversal section of white muscle fibres viewed by scanning microscope. Bar: 33 μm. (c–e) T.E.M. micrographs of the periphery of the white muscle fibres. Bar: 1.36 μm (d). (f) T.E.M. micrographs of contractile myofilaments: the reciprocal arrangement of thick versus thin filaments is observed. W, W*, W**: white muscle fibres with low, moderate and high mATPase activity. MF: muscle fibres; mf: myofibrils; e: endomysium; sr: sarcoplasmic reticulum; mi: mitochondria; n: nucleus; *: muscle fibre to endomysium detachment; ↕: fibre to fibre detachment.

Table 2
Percentages of fibre to fibre detachment, fibre fragmentation, and appearance of intrafibrillar cavities (holes)

Thermal treatment	Fibre to fibre detachment	Fibre fragmentation	Intrafibrillar cavities (holes)
FR	7.27 ± 2.04^{a}	0.87 ± 0.87^{a}	0 ± 0.0^{a}
FC5	88.61 ± 9.86^{b}	40.54 ± 8.45^{b}	0 ± 0.0^{a}
FC10	88.37 ± 9.84^{b}	39.69 ± 5.15^{b}	0 ± 0.0^{a}
ThR	25.07 ± 6.52^{a}	$28.23 \pm 5.35^{a,b}$	$15.02 \pm 8.55^{a,b}$
ThC5	89.31 ± 5.07^{b}	56.96 ± 10.54^{b}	29.52 ± 11.65^{b}
ThC10	99.07 ± 0.70^{b}	55.55 ± 9.46^{b}	42.36 ± 11.0^{b}

Average ± S.E.M. calculated from 50 muscle fibres in each muscle sample from 10 specimens. Different superscripts between rows characterize significant differences ($p < 0.05$).

way (50–75 peripheral intermyofibrillar spaces were evaluated in each one of the 7–10 selected muscle fibres). Besides, the preservation or break of the sarcolemma after thermal treatment was evaluated in these fibres. Values were listed in an Excel 2000 worksheet and then analysed for significant differences (ANOVA, $p < 0.05$) with SPSS (11.0). Tukey-test was used during post-hoc analysis.

3. Results

3.1. Muscle tissue structure and ultrastructure

3.1.1. Fresh raw (FR) muscle samples

By light and scanning microscopy no relevant structural changes were observed in muscle fibres (Fig. 2a, b). They showed a typical polygonal shape and were surrounded by a thin connective tissue layer (endomysium). Occasionally, some fibre to fibre detachments were observed (Table 2). The mATPase reaction of white muscle fibres displayed a typical mosaic appearance. Muscle fibre size was correlated with staining intensity, the smallest fibres showing the highest staining intensity. The apparent structural normality by light and scanning microscopy contrasted with the existence of relevant ultrastructural disorders as revealed by T.E.M (Fig. 2c–f). Such alterations are probably a consequence of a rapid degradation of some organelle after fish death. Many mitochondria and sarcoplasmic reticulum were swollen. Abundant sarcoplasmic reticulum vesicles accumulated among the myofibrils which increased the intermyofibrillar spaces (Table 3). Also, some myofibrils detached from endomysium were observed. Nuclei were also altered, with clumped chromatin and swollen nuclear membrane. However, contractile myofilaments were hardly affected and consequently the typical hexagonal arrangement of thin versus thick contractile filaments showed no alterations.

3.1.2. Fresh cooked (FC5 and FC10) muscle samples

The structural and ultrastructural changes produced by cooking did not extend homogeneously over each complete muscle sample. Whereas some areas of the sample were heavily affected, others hardly showed changes. This determined high values for the standard error of the mean in the quantified parameters (Tables 2 and 3), which influenced the statistical significance of differences.

The selected images in Figs. 3 and 4 were chosen so as to describe the most relevant and usual changes after cooking for 5 and 10 min, respectively. Cooking for 5 min produced important muscle tissue changes. Muscle fibres as well as myofibrils showed broad clear spaces among them (Fig. 3a, b, e). Such spaces were occupied by liquids and produced by shrinkage of muscle fibres, as a consequence of thermal protein coagulation. The interstitial tissue contained a slight mesh of amorphous material, which partly may correspond to gelificated collagen (Fig. 3a–c). However, some remains of collagen fibres could still be observed. Another important feature was the appearance of abundant dense, granular aggregates mainly located

Table 3
Percentage of myofibrils detached to endomysium, increased intermyofibrillar spaces and sarcolemma break

Thermal treatment	% Myofibrils detached to endomysium	% Increased intermyofibrillar spaces	% Sarcolemma break
FR	5.35 ± 0.73^{a}	18.03 ± 3.86^{a}	3.33 ± 7.45^{a}
FC5	93.08 ± 2.74^{b}	69.18 ± 3.69^{b}	93.33 ± 6.67^{b}
FC10	92.48 ± 2.48^{b}	63.15 ± 4.27^{b}	100 ± 0.00^{c}
ThR	20.74 ± 4.57^{a}	51.36 ± 4.4^{b}	41.9 ± 18.28^{b}
ThC5	96 ± 13.15^{b}	53.44 ± 4.9^{b}	97.78 ± 2.22^{b}
ThC10	86 ± 2.74^{b}	61.68 ± 4.79^{b}	100 ± 0.00^{b}

Average ± S.E.M. from 7 to 10 muscle fibres in each muscle sample from 5 specimens. Different superscripts between rows characterize significant differences ($p < 0.05$).

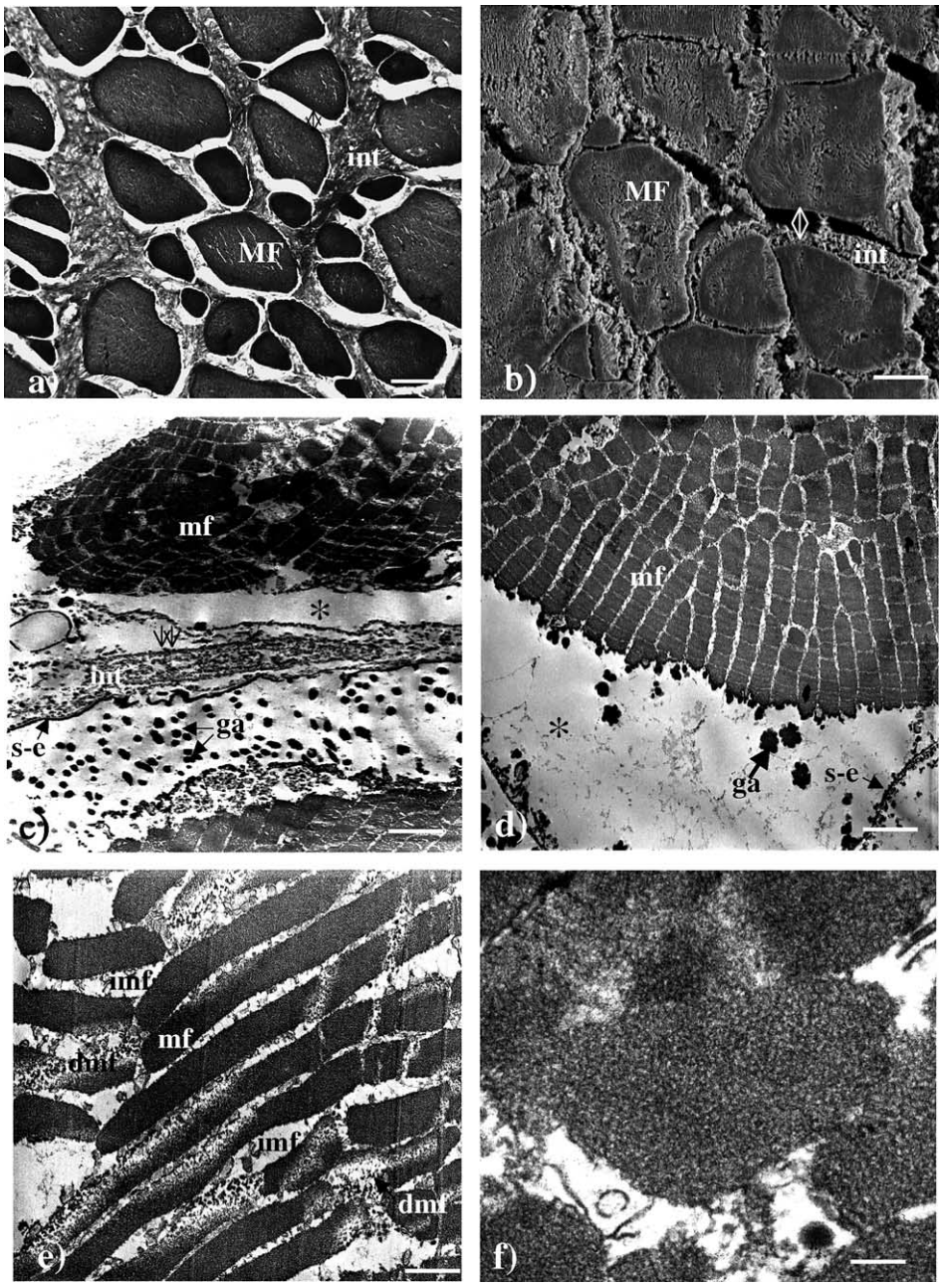

Fig. 3. White muscle samples after cooking for 5 min (FC5). (a) Haematoxylin/Eosin staining. Bar: 84.4 μm. (b) Scanning micrograph, bar: 32 μm (c–f) T.E.M. micrographs. Bars: 3.27 μm (c, d), 1 μm (e), 0.2 μm (f). MF: muscle fibres; mf: myofibrils; int: interstitial material; note remnants of collagen strands in (c) (arrows); s-e: coagulated sarcolemma and endomysium; ga: granular aggregates; imf: intermyofibrillar space; dmf: disintegrated myofibrils; *: myofibrils to endomysium detachment; ↕: fibre to fibre detachment.

at the subsarcolemmal space (Fig. 3c, d). Such aggregates might be produced by disintegration of sarcoplasmic, sarcolemmic and contractile coagulated proteins. Cooking also caused the extensive break of sarcolemmas and coagulation of endomysium. Consequently, the limits of individual muscle

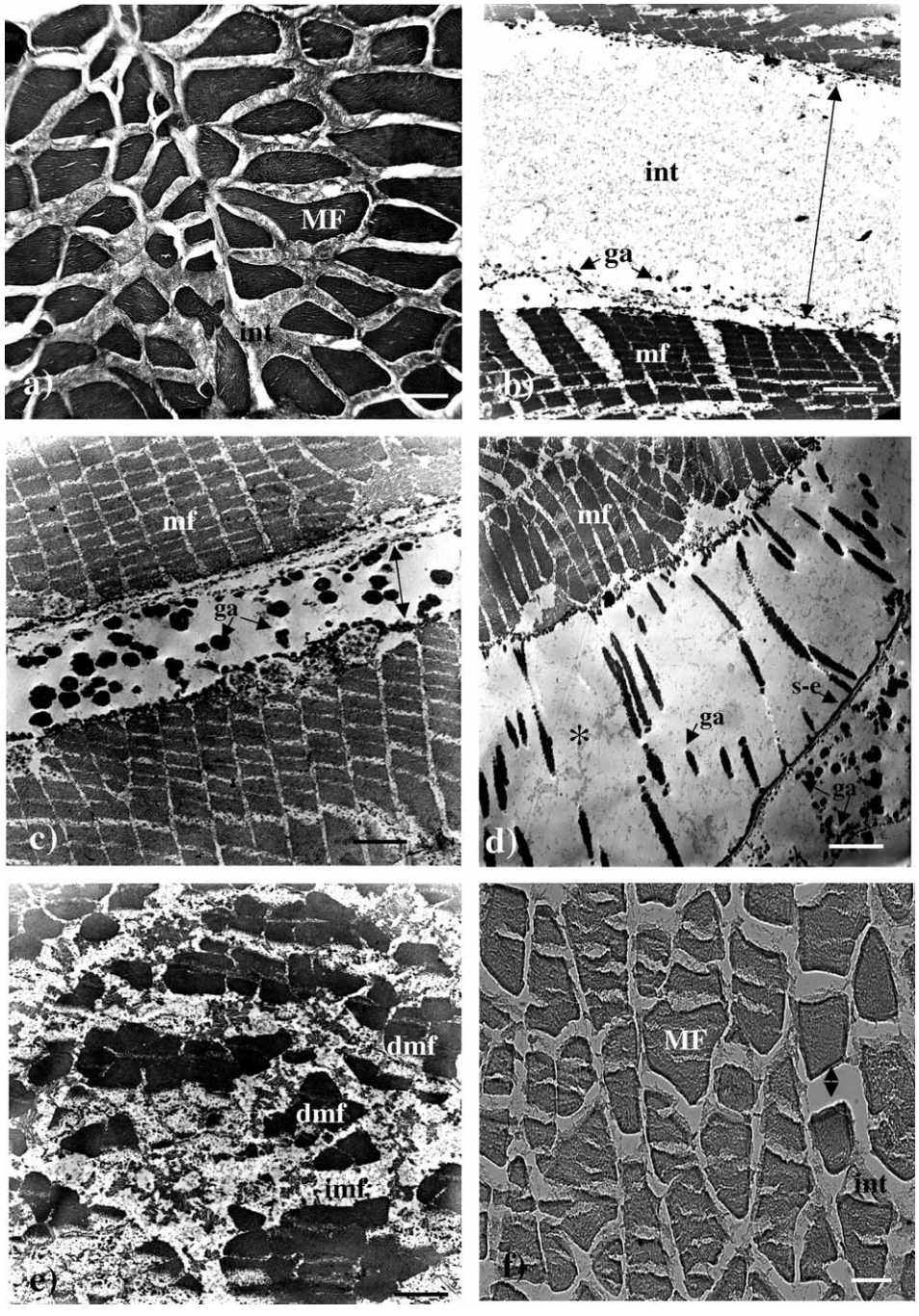

Fig. 4. White muscle samples after cooking for 10 min (FC10). (a) Haematoxylin/Eosin staining. Bar: 90 μm. (b–e) T.E.M. micrographs. Bars: 3.2 μm (b), 2.05 μm (c), 3.2 μm (d), 2.05 μm (e). (f) mATPase staining after acid preincubation pH 4.6 (30 s). No mosaic staining of white muscle fibres was observed. Bar: 50 μm. MF: muscle fibres; mf: myofibrils; int: interstitial material; ga: granular aggregates; s-e: coagulated sarcolemma and endomysium; dmf: disintegrated myofibrils; imf: intermyofibrillar space; *: myofibrils to endomysium detachment; ↕: fibre to fibre detachment.

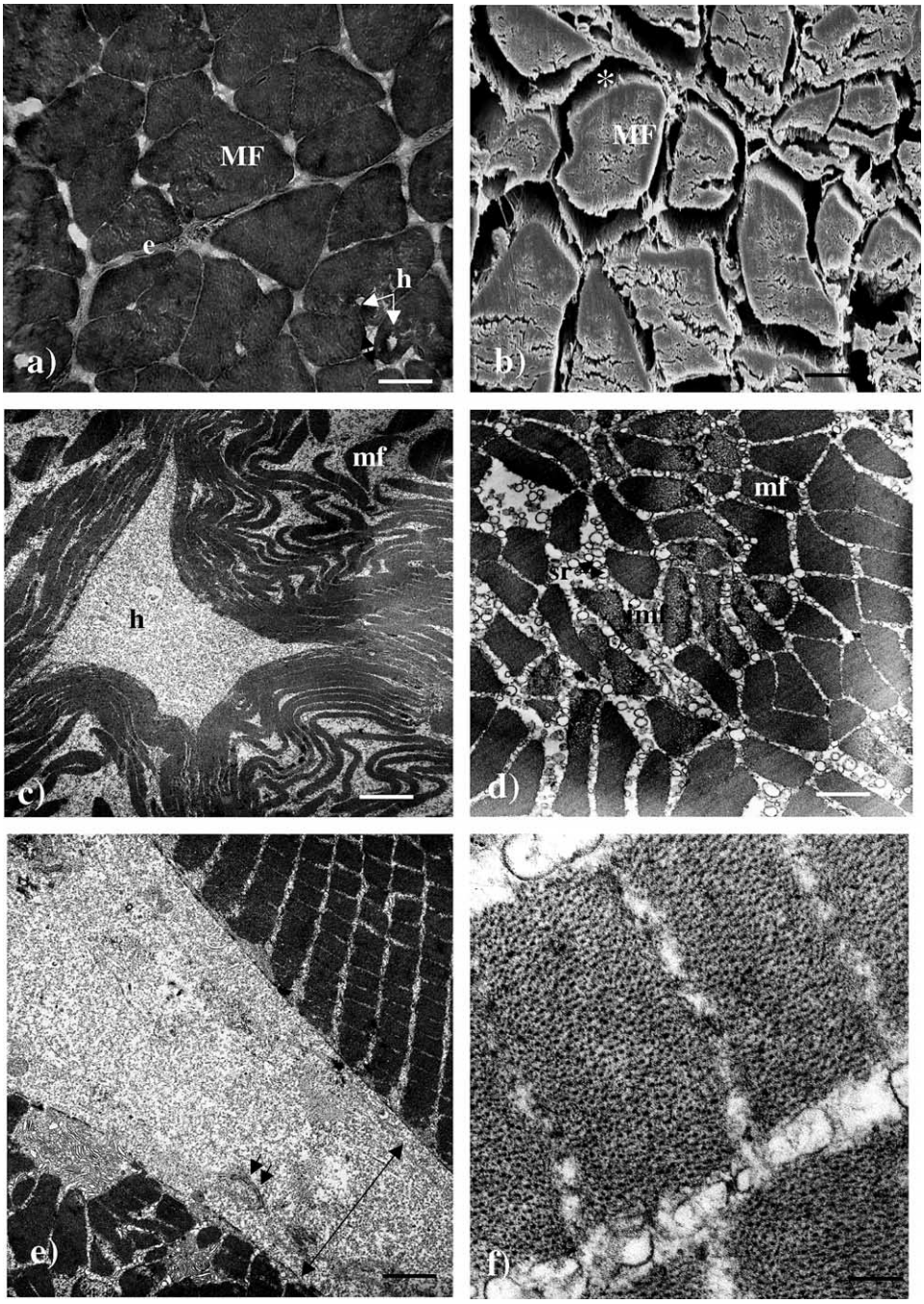

Fig. 5. Thawed raw muscle samples (ThR). (a) Haematoxylin/Eosin staining. Bar: 76 μm. (b) Scanning micrograph. Bar: 32.36 μm. (c–f) T.E.M. micrographs. Bars: 2.62 μm (c), 3.27 μm (d), 1.76 μm (e) and 0.14 μm (f). Note the loss of the pattern of arrangement between thick and thin contractile filaments in (f). MF: muscle fibres; mf: myofibrils; e: endomysium; h: hole (intrafibrillar cavity); sr: sarcoplasmic reticulum; imf: intermyofibrillar space; *: myofibrils to endomysium detachment; ↕ fibre to fibre detachment; ↓↓: collagen strands.

fibres were defined by an electron dense line of amorphous material (Fig. 3c, d). Thermal coagulation of contractile material determined the loss of both the mATPase activity of muscle fibres and the typical spatial arrangement of contractile filaments (Fig. 3f). Besides, sarcoplasmic organelles such as

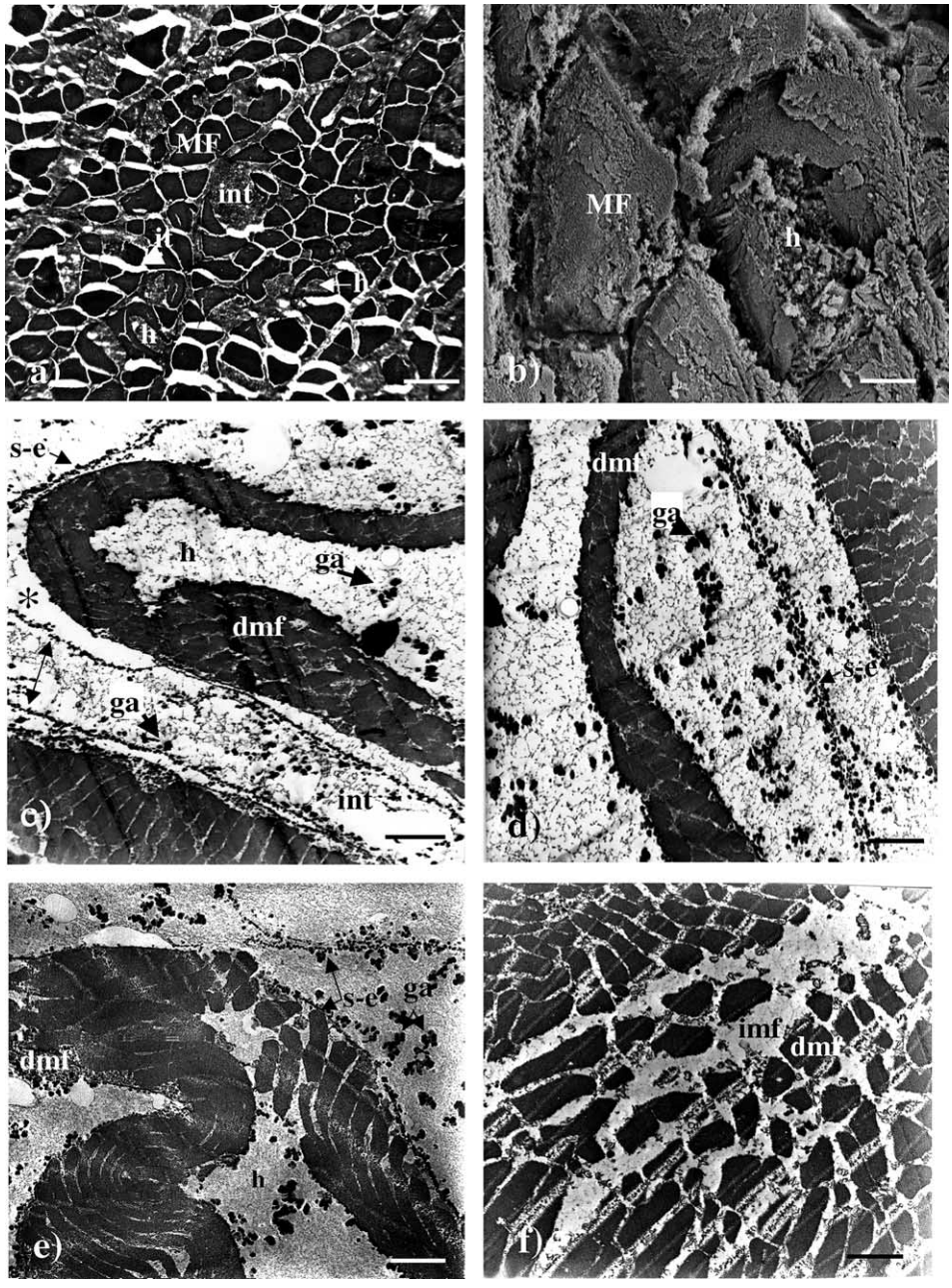

Fig. 6. Thawed and cooked white muscle samples (ThC). (a, b, e, f) 10 min cooking and (c, d) 5 min cooking. (a) Haematoxylin/Eosin staining. Bar: 165 μm. (b) Scanning micrograph. Bar: 32.36 μm. (c–f) T.E.M. micrographs. Bars: 1.97 μm, 3.2 μm, 2.6 and 1.97 μm, respectively. MF: muscle fibres; h: hole (intrafibrillar cavity); int: interstitial material; dmf: disintegrated myofibrils; s-e: coagulated sarcolemma and endomysium; imf: intermyofibrillar space; ga: granular aggregates; *: myofibrils to endomysium detachment; ↕: fibre to fibre detachment.

mitochondria, sarcoplasmic reticulum and nuclei were seriously damaged or destroyed.

After cooking for 10 min, most morphological features were quite similar to that found after cooking for 5 min (Fig. 4). However, no more remnants of collagen fibres could be observed after cooking for 10 min and most myofibrils appeared highly disintegrated or broken.

3.1.3. Thawed raw (ThR) muscle samples

The structural changes determined by freezing for 24 h were also not uniform over the samples. Thus, some areas of the samples were significantly more affected than others, this also influencing the standard error of the quantified parameters and the statistical significance of the results (Tables 2 and 3) The more common structural finding in thawed muscle tissue was the appearance of abundant clear spaces produced by the formation of ice crystals. Such clear spaces were observed among the muscle fibres as well as at the subsarcolemmal space (Fig. 5a, b). Also, some muscle fibres presented intrafibrillar cavities (holes) (Fig. 5c). Most muscle fibres showed shrinkage, together with focal water content changes over the muscle samples. The sarcoplasmic reticulum was disrupted and swollen (Fig. 5d), what caused a significant increasing of the intermyofibrillar spaces (Table 3). The structure of myofibrils was not uniformly affected, but the mATPase activity almost totally lost. Also, the reciprocal arrangement of contractile filaments was altered (Fig. 5f). However, the connective tissue of the endomysium was not strongly affected, thus collagen fibres and sarcolemmas could be observed (Fig. 5e).

3.1.4. Thawed and cooked (ThC5 and ThC10) muscle samples

The structural changes in these samples were quite similar to those in the fresh cooked muscle samples. In addition to the structural changes determined by cooking, the most relevant change in ThC samples was the presence of big intrafibrillar cavities (holes) in many muscle fibres (Fig. 6a–e) (Table 2). Such cavities were occupied by liquids, granular aggregates and a mesh of amorphous material consequent to disintegration of contractile and sarcoplasmic coagulated proteins. Also, coagulation of sarcolemmas and endomysium was relevant, both of them replaced by dense “chain like” lines of small granular aggregates (Fig. 6c–e) (Table 3). Coagulation and disintegration of myofibrils produced large amounts of dense aggregates and abundant amorphous material among and inside the damaged muscle fibres (Fig. 6c–f).

3.2. Morphometric measurements and quantification of changes

Values for white muscle fibre diameters and fibre density in FR muscle samples have been summarized

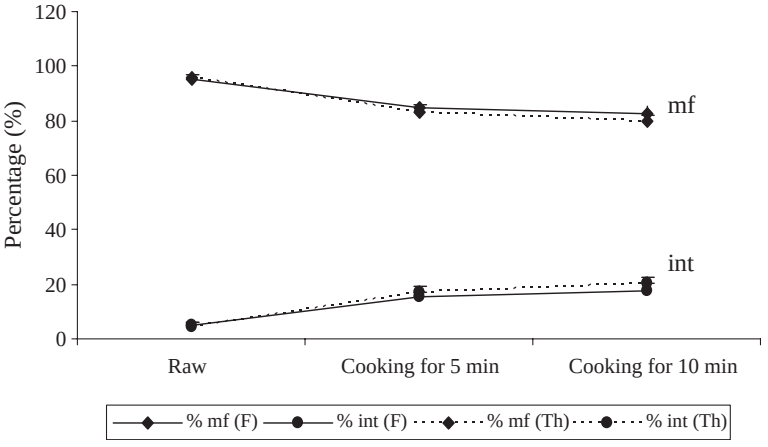

Fig. 7. Percentages of myofibrillar (% mf) and interstitial (% int) materials in white muscle samples, processed in raw state or after cooking for 5 or 10 min from fresh (F) or thawed samples (Th). Average values ± S.E.M. from 10 specimens.

in Table 1. These parameters showed important variation according to differences in size and weight of specimens. The minimum and maximum average values for the white muscle fibre diameters were 72 ± 3.18 μm and 119.72 ± 6.05 μm, respectively. Thermal treatments caused different degrees of water loss consequent to muscle fibre shrinkage. In ThR samples water loss was of $1.37 \pm 0.1\%$ and muscle fibre shrinkage $8.4 \pm 5.3\%$ ($p > 0.05$). However, in FC samples water loss increased significantly to 13.72 ± 2.04 and 14 ± 1.52 after cooking for 5 and 10 min, respectively, with a corresponding muscle fibre shrinkage of $7.48 \pm 6.6\%$ and $10.28 \pm 7.3\%$ ($p > 0.05$). Percentages of myofibrillar material in muscle samples were also reduced by cooking, whereas the interstitial material significantly increased ($p < 0.05$) (Fig. 7). A slight correlation between the percentage of interstitial material and muscle fibre size was observed. Thus, specimens with higher muscle fibre densities showed higher percentages of interstitial material after cooking for 5 min (Fig. 8), although this correlation was not significant ($p = 0.051$).

Results for quantification of structural and ultrastructural parameters are shown in Tables 2 and 3. As explained above, the effects of thawing or cooking on muscle samples were not uniform over the muscle samples. Hence, values for the quantified parameters showed high variation among the randomly selected muscle fibres. Concerning the FR samples, the high values of SEM may be due to individual variation among the sampled specimens in the degree of pre-rigor degradation when the muscle samples were fixed (3 h postmortem). As a general rule, structural and ultrastructural muscle disorders were low or absent in FR samples, medium in ThR samples and maximum in cooked samples (FC and ThC). The most relevant changes in FR samples were the appearance of some fibre to fibre detachments and the presence of many increased intermyofibrillar spaces (18% average per fibre). Cooking length (5 or 10 min) did not caused significant differences in both FC and ThC muscle samples. The freezing/thawing process caused the appearance of intrafibrillar holes inside 15.02% of muscle fibres in ThR samples. This value was higher, but not significant in ThC5 and ThC10 muscle samples.

4. Discussion

4.1. Fresh raw (FR) and thawed raw (ThR) muscle samples

After death, fish pass through several stages: rigor mortis, degradation, autolysis and bacterial spoilage (Sigholt et al., 1997). The onset of rigor mortis in unstressed fish is commonly observed 6–24 h postmortem but the structural and ultrastructural disorders of postmortem degradation can be microscopically observed even at pre-rigor condition (Liljemark, 1969). In the present work, the axial musculature of sea bass after 3 h postmortem on ice (pre-rigor) showed unequivocal evidence of early structural and ultrastructural degradation. Muscle changes affected the fibre to fibre attachment (7.27% in FR muscle samples), the myofibre to endomysium attachment (5.35%), as well

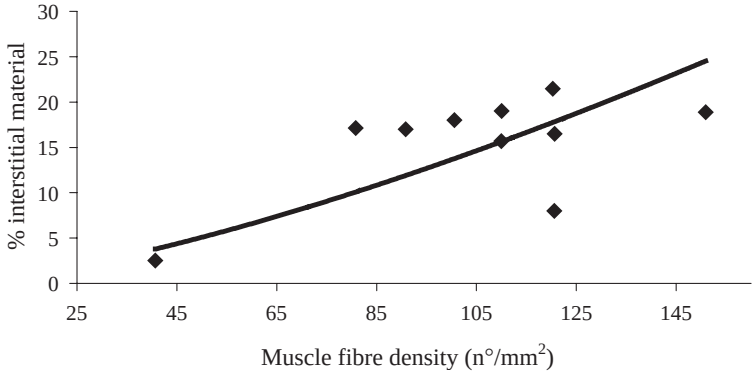

Fig. 8. Relationship between the density of white muscle fibres and the percentage of interstitial material in muscle samples after 5 min cooking. Power trend: $y = 0.0193x^{1.4247}$, $r^2 = 0.62$.

as the mitochondria and sarcoplasmic reticulum, which in many cases appeared swollen. Swelling of the sarcoplasmic reticulum caused an increase of the intermyofibrillar spaces (18% average per fibre). Mitochondrial and sarcoplasmic reticulum swelling may be explained by changes in the sarcolemmal permeability to liquids, consequent to a rapid decrease of muscle pH during pre-rigor state (Hegarty et al., 1978). The increased input of water into the muscle fibres determines the appearance of water vesicles within the sarcoplasm and swelling of membranous organelle such as mitochondria and sarcoplasmic reticulum. Changes in the nuclei affecting the normal appearance of the nucleoli, chromatin and nuclear membrane, as described in this study, are also very common during initial necrosis of muscle tissue (Armiger et al., 1976; Collan and Salmenperä, 1976). To explain the moderate fibre to fibre and myofibrils to endomysium detachments observed in FR samples (Tables 2 and 3), it is likely to consider the onset of local proteolytic activity at the level of both the extracellular matrix (Bremmer and Hallet, 1985; Ando et al., 1991) and costameres (Papa et al., 1997; Taylor et al., 2002). As demonstrated in the blue grenadier (*Macruronus novaezelandiae*) degradation of the extracellular matrix by endogenous collagenases and/or other proteinases was responsible of progressive detachment of the muscle fibres to myocommata (Bremmer and Hallet, 1985). Besides, in the sea bass degradation of dystrophin, tytin and alpha-actinin resulted in sarcolemma detachment from myofibrils in costameres (Papa et al., 1997). Particularly, dystrophin which is located in costameres was quickly degraded by 50% within 24 h postmortem. In Atlantic salmon, detachment of myofibrils to endomysium has been shown to decrease texture of fish fillets significantly after 24 h of postmortem ice storage (Taylor et al., 2002). Additionally, a progressive myofibre detachment from the myocommata was associated to loss of rigor stiffness by 5 days postmortem. On the basis of these precedents it is suggested that further investigations aimed to define how muscle structural degradation correlates with textural parameters in sea bass fillets are required for a better understanding of the factors determining flesh quality in this farmed species.

Concerning the contractile thick and thin myofilaments in FR muscle samples we did not observe relevant alterations but preservation of the typical hexagonal arrangement of thin versus thick myofilaments. A limitation of the present study was the lack of longitudinally sectioned muscle samples. Thus, important muscle features such as the pattern of bands in sarcomeres and attachments of myofibrils to myocommata could not be evaluated. Some previous works focused on this subject were Papa et al. (1997) and Taylor et al. (1997). As it was described in these studies on sea bass and also in Atlantic salmon (Taylor et al., 2002) fish myofibrils are very stable to postmortem degradation and does not undergo the rapid early proteolytic activity described in mammals (Taylor et al., 1995).

Our results in sea bass indicated that the onset of ultrastructural changes of the muscle tissue after fish death was very rapid. This may be related to both the velocity of decrease and final pH values of the fillet. Ofstad et al. (1996a) studied the ultrastructure of muscle fibres in farmed cod and salmon and in wild cod stored in ice for 3 h. Farmed specimens underwent a more rapid and severe postmortem flesh degradation than wild cod which could be produced by a lower than normal muscle pH in farmed fish. In sea bass, we have recently found lower muscle pH values at rigor state in farmed than in wild specimens (6.45 and 6.75, respectively) (unpublished results). Thus, lifestyle and/or nutritional status influence the postmortem muscle pH value of the flesh and this may be related with the onset of the postmortem structural alterations.

Freezing as well as the subsequent frozen storage is known to influence the appearance of structural flesh alterations (Fauconneau et al., 1995; Hall, 2001). In the present work ThR muscle samples of sea bass showed higher ultrastructural modifications than FR samples kept in ice for 3 h. Thus, the formation of ice crystals during freezing produced abundant interfibrillar spaces, intrafibrillar fragmentation (Table 2), and alterations on the contractile apparatus (Fig. 5). Similar structural features have been described in detail by Bello et al. (1982) in the goldfish (*Carassus auratus*, L.). In parallel to the formation of ice crystals, a high concentration of salt surrounding the muscle fibres is produced, which determines a dehydrating action on the cells (Cheftel and Cheftel, 1980; Hall, 2001). This fact may explain the shrinkage observed in the muscle fibres of sea bass after thawing and the subsequent

loss of water in thawed samples. Another factor influencing the structural changes in frozen flesh is the velocity of freezing, such that the denaturalization of muscle proteins increases when freezing at slow rate and when the final temperature is not very low (Cheftel and Cheftel, 1980; Hall, 2001). Both conditions occurred in our study, and were responsible of moderate percentages of fibre to fibre detachment and myofibrils detached to endomysium which are associated to protein denaturation (Tables 2 and 3).

4.2. Fresh cooked (FC) and thawed cooked (ThC) muscle samples

The thermal treatment significantly changed the muscle tissue structure and ultrastructure of sea bass. However, no significant differences associated to cooking length (5 or 10 min) were observed between the quantified parameters. Only, in the FC5 muscle samples some remnants of collagen and moderate amount of amorphous material occupying the interfibrillar spaces were observed, whereas in the FC10 samples the collagen fibrils were entirely lost, and interstitial spaces appeared completely filled up with amorphous material. The interstitial material after cooking is supposed to be formed by sarcoplasmic proteins that are released and coagulated in the interfibrillar spaces, as well as by gelification of the connective tissue (Hatae et al., 1990; Fauconneau et al., 1995). Interstitial material has been correlated with the diameter of the muscle fibres and with the texture of the flesh (Hatae et al., 1984, 1990; Hurling et al., 1996). Thus, species with firmer texture had thinner muscle fibres with considerable heat-coagulating material between them, while species having soft texture had thick muscle fibres with little surrounding heat-coagulating material. In our study, cooking for 5 min produced a slightly higher percentage of interstitial material in specimens with smaller fibres (higher muscle fibre density) (Fig. 8). In order to increase the knowledge in this subject, we are now studying the relationship between muscle fibre density and some textural parameters in cooked flesh of sea bass.

One common finding in cooked samples was the appearance of abundant electron dense aggregates at the subsarcolemmal space as well as at the interfibrillar spaces. These aggregates mainly corresponded to denaturated and coagulated myofibrillar proteins, as described in muscle samples of farmed cod and salmon when heated at 60 °C (Ofstad et al., 1996b). Besides, these aggregates may also be formed by coagulation of sarcoplasmic and sarcolemmic proteins.

In addition to the typical muscle changes determined by cooking, in ThC muscle samples the most significant result was the appearance of abundant intrafibrillar cavities (holes) containing liquids, amorphous material and abundant granular aggregates. This may indicate a higher disintegration of both the contractile and sarcoplasmic material in ThC than in FC muscle samples. Intrafibrillar cavities are supposed to be produced by the formation of ice crystals during the freezing process and also by the thermal treatment. Intrafibrillar cavities were also described by Ofstad et al. (1996b) in heated muscle samples (60 °C) of fed cod and salmon. These authors did not find such cavities in heated muscle samples of wild cod, what was related to a different state of postmortem degradation in farmed and wild fish prior to heating.

Cooking also caused a massive break of sarcolemmas, which were replaced by an electron dense line of coagulated amorphous material joined to the endomysium. In ThC muscle samples sarcolemma and endomysium were even replaced by a “chain like” dense line of small granular aggregates. These results resemble those of Ofstad et al. (1996b) in reared cod, where sarcolemma of heated muscle was ruptured and electron dense aggregates were seen as a string of pearls along the periphery of the fibres.

5. Conclusion

The initial state of muscle tissue (fresh or thawed) as well as the thermal treatments notably influenced the structure and ultrastructure of the muscle tissue of sea bass. In FR muscle samples the observed structural changes corresponded to typical early postmortem processes which in farmed sea bass started quite rapidly. The ThR samples displayed moderate muscle disorders which were mainly associated to the formation of ice crystals during freezing and to protein denaturation. Cooking largely altered the muscle tissue structure and ultrastructure due to thermal coagulation of proteins and associated changes in water content. The tissue changes produced by the thermal

treatment were slightly increased when the muscle samples had been previously frozen.

Based upon our results, it is likely to think that the structural changes in the muscle tissue of sea bass produced by postmortem degradation, thawing and/or cooking should modify the textural and organoleptic characteristics of the flesh. Further studies in this sense are necessary to attain a better understanding of all these factors.

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