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Muscle tissue structure and flesh texture in gilthead sea bream, *Sparus aurata* L., fillets preserved by refrigeration and by vacuum packaging

María Dolores Ayala [a,*], Marina Santaella [b], Carmen Martínez [b], María Jesús Periago [b], Alfonso Blanco [c], José María Vázquez [a], Octavio López Albors [a]

[a] Department Anatomía y Anatomía Patológica Comparadas, Facultad de Veterinaria, Universidad de Murcia, 30100, Spain
[b] Department Tecnología de Alimentos, Nutrición y Bromatología, Facultad de Veterinaria, Universidad de Murcia, 30100, Spain
[c] Department Anatomía y Anatomía Patológica Comparadas, Facultad de Veterinaria, Universidad de Córdoba, Spain

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ABSTRACT

Fillets were analysed from 66 gilthead sea bream specimens (*Sparus aurata* L). Thirty-six fillets were refrigerated (2 °C). The rest of the fillets were vacuum-packed and refrigerated. Muscle and sensorial parameters were evaluated for 22 days post-mortem.

Textural parameters were higher in early stages. The refrigerated fillets had the lowest values. Most of textural parameters showed a negative correlation with the detachments among fibres in both groups.

Ultrastructural results in vacuum-packed fillets showed that sarcolemma–endomysium was gradually disrupted, with almost a complete loss at 22 days. Initially, the detachments of myofibrils from the sarcolemma–endomysium were scarce. Mitochondria and sarcoplasmic reticulum were swollen from the first stages onwards. From 16 days onwards, the intra-cytoplasmic organelles were significantly altered and the smooth reticulum appeared hypertrophied, indicating an increase of the autophagic mechanisms. Sarcomeres were gradually altered, mainly at the I-band level, which showed a loss of actin filaments and Z-line disruptions from 12 to 16 days onwards.

In non-vacuum fillets the muscle tissue was already markedly altered from the early stages. From five days onwards, the detachments of myofibrils from the sarcolemma were frequent and the hypertrophied smooth reticulum and the degraded lysosomes were already observed.

1. Introduction

Deterioration of fish flesh results from the combination of physical, chemical, biochemical and microbial processes (Delbarre-Ladrat, Chéret, Taylor, & Verrez-Bagnis, 2006). Endogenous enzymes (calpains, cathepsins, etc.) promote proteolysis of the cytoskeletal components, with the degradation of the myofilaments and of the costameres that link sarcomeres with the sarcolemma (Delbarre-Ladrat et al., 2006; Ofstad et al., 1996). These alterations have been related to the loss of hardness in fish fillets by the cited authors. In a recent work, we studied the muscle tissue degradation in gilthead sea bream stored in refrigeration, and we found a correlation between muscle fibre detachment and the loss of textural parameters (Ayala et al., 2010).

When the post-mortem degeneration takes place, the degradation of proteins creates ideal conditions for the growth of microorganisms (Cheret, Chapleau, Delbarre-Ladrat, Verrez-Bagnis, & De Lamballerie, 2005). These microorganisms make food organoleptically unacceptable for consumption because of changes in colour, odour and texture (Özogul, Polat, & Özogul, 2004). Inhibition of these microorganisms increases the potential shelf life of the flesh.

The method of preservation is important in order to delay the degradation. The modification of the atmosphere (MAP) within the package by means of an adequate percentage of carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2) produces important bacteriostatic effects that increase the useful shelf life of the fish (Gibson & Davis, 1995). Other processes, e.g., filleting or vacuum packaging, also increase the useful shelf life or maintain the quality of the products longer (Huss, 1999). Thus, filleting implies the evisceration of the fish and it eliminates an important source of intestinal microorganisms. Vacuum packaging decreases the supply of oxygen to the aerobic bacteria in the flesh. In addition, this latter method improves the stability of the meat during chilled storage by reducing the rancidity of fats.

There are some studies about the effects of filleting and vacuum packaging fish, but they are still scarce. Using sea bream, Chouliara, Savvaidis, Panagiotakis, and Kontominas (2004) studied the

* Corresponding author. Tel.: +34 968 398793; fax: +34 968 364798.
E-mail address: mdayala@um.es (M.D. Ayala).

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preservation of vacuum-packaged fillets by irradiation. Also, Özogul et al. (2004) studied vacuum packaging fillets from sardines (*Sardine pilchardus*). These authors studied the changes in chemical, sensory and microbiological parameters, but the muscle tissue structure was not studied in the cited works. In fact, there are few studies about changes in muscle tissue structural in fish after filleting or vacuum packaging; for example, Bahuaud et al. (2008) studied the muscle histology in Atlantic salmon (*Salmo salar, L.*) pre-rigor fillets. The structural and ultrastructural changes, however, have not been studied in refrigerated and vacuum-packaged fillets of *Sparus aurata* throughout their post-mortem degradation. The aim of this study is to determine the muscle tissue degradation and the evolution of the textural parameters in refrigerated and vacuum-packed fillets of this species for 22 post-mortem days. The results for the present study are compared with the results for refrigerated, whole un-filleted fish (Ayala et al., 2010).

2. Materials and methods

2.1. Fish samples and sample treatment

Sixty-six specimens of fresh sea bream were obtained from a fish market in Alcantarilla (Murcia, Spain) at the commercial size (≈30 cm total length, ≈410 ± 5g weight) within one day of harvesting. The fish were delivered to the Veterinary Faculty of Murcia in sealed ice-pellet-filled cases. The fish were beheaded, gutted and filleted (100 ± 5g each fillet). All the fillets were obtained from the left half of the axial musculature of each fish. Thirty of them were vacuum-packed (VF lot) and refrigerated at 2 °C in order to analyse the following post-mortem days: 1, 7, 12, 16 and 22. The other 36 fillets were preserved by refrigeration (RF lot) and analysed at 0, 1, 5, 10, 15 and 22 post-mortem days.

The fillets from VF lot were put in Polyethylene/Polyamide (PE/PA) barrier pouches, 100 μm thick, with an oxygen permeability of 50 $cm^3m^{-2}d^{-1}bar^{-1}$ and a water vapor permeability of 2.3 g m^{-2} d^{-1} ($T = 23$ °C, RH = 0%). They were vacuum sealed using an ORVED Professional Family vacuum sealer (ORVED srl unipersonale Italy) with a final vacuum level of 0.15 bar. Both RF and VF lots were iced immediately with flake ice at a fish:ice ratio of 1:1 in an insulated high-density polyethylene box at 2 °C. When necessary, more ice was added.

2.2. Muscle sample processing for instrumental textural measurements and pH

The texture profile analysis method (TPA) was used to evaluate the texture based on the compression of the sample with a TA-XT2 texture analyzer (Stable Microsystems, Surrey, England) equipped with a load cell of 25 kg and Texture Pro v. 2.1 software. A flat-ended cylinder of 20 mm diameter was selected to simulate the human finger. A portion 1.5 cm thick, 5 cm long and 2.5 cm high was obtained from the dorsal musculature of the left fillet. The measurement was composed of two consecutive 50% compressions of the sample perpendicularly to the muscle fibres' orientation at a crosshead speed of 50 mm/min. Force by time data from each test was used to calculate the values for the TPA parameters as described by Bourne (1978): hardness (peak force of the first compression cycle), gumminess (hardness multiplied by cohesiveness), adhesiveness (negative force area under the baseline between the compression cycles), cohesiveness (ratio of positive force area during the second compression compared to that during the first compression), chewiness (hardness multiplied by cohesiveness multiplied by springiness) and springiness (height that the food recovers during the time elapsing between the two compression cycles). All measurements were taken at room temperatures (22–23 °C).

The pH was measured by dispensing 10g of homogenized fish muscle in distilled water (1:5 w/v) using a glass electrode digital pH meter (Crison Micro-pH2000, Alella, Spain).

2.3. Muscle samples processing for structural and ultrastructural studies

Muscle samples were obtained by removal of one 0.5 cm thick piece of the caudal third of the fillet from the cross-section of the musculature, caudally to the anal opening, according to the methodology described by Ayala et al. (2005, 2010). The samples were trimmed into 5 × 5 × 5 mm blocks. Half of the blocks were frozen in 2-methylbutane (−80 °C) snap frozen over liquid nitrogen. Sections 8 μm thick were obtained at −20 °C in a cryostat and stained with Haematoxylin/Eosin to perform structural studies by light microscopy. The remaining blocks were processed for transmission electron microscopy (TEM). The TEM blocks were trimmed and fixed in 2.5% glutaraldehyde in buffered 0.1 M cacodylate (pH 7.2–7.4) for 3 h, at 4 °C. More TEM processing was performed in the Microscopy Service of the University of Murcia, according to standard protocol for epoxy embedding. The ultrathin sections were viewed using Zeiss EM 109 and EM 10C (München, Germany) transmission electron microscopes at 80 kV.

Morphometric analysis was carried out using five to six specimens per stage in both groups by means of an image analysis device (Qwin, Leica) connected to a light photomicroscope (Leitz Dialux, 20). The percentages of fibre-to-fibre detachments (fibres with loss of interfibrillar adhesion) and intrafibrillar breaks (fibres with transversal internal fragmentation) were calculated in all the stages at a minimum of 150 fibres/fish, according to methodology used with salmon by Taylor, Fjaera, and Skjervold (2002), in sea bass, *Dicentrarchus labrax* L., by Ayala et al. (2005) and in gilthead sea bream by Ayala et al. (2010). Ultrastructural parameters were studied by direct TEM viewing of six specimens per stage in both groups. In each specimen, a minimum of seven to ten fibres were observed in transversal and longitudinal samples, as carried out by Ayala et al. (2010).

2.4. Statistical analysis

Values were analysed for significant differences (ANOVA, $^*P < 0.05$) with SPSS (15.0 version) for both groups. A Tukey-test was used during post-hoc analysis. Both groups were compared with the values obtained by Ayala et al. (2010) for refrigerated whole gilthead sea breams (RW lot) in order to elucidate the effect of the processing and time of storage on the different groups. Mean values ± SEM were obtained for each group. Data analyses also included a 2-way ANOVA test to calculate the effects of the type of treatment and post-mortem sampling days on the texture parameters' profiles and pH measurements. To measure the strength of the relationships between structural and textural parameters, a correlation analysis using the Pearson's correlation coefficient was conducted.

3. Results

3.1. Textural parameters and pH

The results of the two-way ANOVA test are shown in Table 1 to ascertain the effects of the type of preservation treatment and time of storage on texture parameters and the pH of the flesh. The influence of both factors on each variable tested was clear with P values <0.001, except for cohesiveness, which had no variation during storage on ice.

Table 2 shows the textural parameters throughout all post-mortem stages for VF and RF, as well as in RW (Ayala et al., 2010) in order to compare all the groups. As shown, the highest values of texture were found during the first post-mortem days, mainly in