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Biochemical changes and quality loss during chilled storage of farmed turbot (*Psetta maxima*)

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Abstract

Changes in three of the major biochemical components – nucleotides, lipids and proteins – related to quality loss in farmed turbot, were determined during 29 days of iced storage; results were complemented with sensory analysis. Nucleotide degradation, as estimated by the *K* value, underwent a gradual increase until day 19, in agreement with the loss of freshness observed for the sensory scores (high quality: days 0–2; good quality: days 3–14; fair quality: days 15–19). After day 19, the fish was judged unacceptable and the *K* value did not show differences until the end of storage. Lipid hydrolysis and oxidation occurred at slow rates, free fatty acid contents and the peroxide value being below 20.0 g kg^{-1} lipids and 4.00 meq active oxygen kg^{-1} lipids, respectively, during the whole storage. The content of fluorescent compounds did not increase significantly until day 19, when a sharp increase was detected. The electrophoretic protein profiles of turbot muscle did not point to any major protein degradation event or any significant change in protein during storage. However, a new band, corresponding to 22 kDa, could be observed at day 2 in the low-ionic strength buffer extract, whose concentration seemed to increase at days 9 and 14 and was present until the end of the chilled storage. The results obtained in this work indicate slow and gradual biochemical changes and long shelf life and good quality times (19 and 14 days, respectively) for iced turbot; these long times would be very profitable when turbot commercialisation is carried out in places distant from production farms.

Keywords: Turbot; Aquaculture; Chilling; Nucleotides; Lipids; Proteins; Quality

1. Introduction

Seafood products have attracted considerable attention as a source of high amounts of important nutritional components to the human diet (Ackman, 1989; Piclet, 1987). However, in recent years the fishing sector has suffered from dwindling stocks of traditional species as a result of dramatic changes in their availability. This has prompted fish technologists and the fish trade to pay more attention to aquaculture techniques as a source of fish and other seafood products (FAO, 2000; Josupeit, Lem, & Lupin, 2001).

Assurance of both the quality and safety of seafood will be a major challenge faced by humankind in this new century. In this sense, wild and farmed fish species are known to deteriorate after death due to the action of different mechanisms (Hsieh & Kinsella, 1989; Pigott & Tucker, 1987). During fish chilled storage, biochemical changes are known to take place, such as changes in the protein and lipid fractions and the formation of amines (volatile and biogenic) and hypoxanthine. As a consequence of these events, a deterioration in sensory quality, a loss of nutritional value, and negative modifications of the physical properties of fish muscle have been reported (Bennour, El Marrakchi, Bouchriti, Hamama, & El Ouadaa, 1991; Nunes, Batista, & Morâo de Campos, 1992; Olafsdóttir et al., 1997).

Turbot (*Psetta maxima*, also known as *Scophthalmus maximus*) is a flat fish species of high commercial value found in Northern waters and widely appreciated for its firm, white, and flavourful flesh. In recent years, the

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increasing production of this species as an aquaculture product has made it more available to consumers, when the wild product is increasingly less consumed because of its low availability and high cost. Extensive work has been carried out on the effects of diet on turbot growth (Cáceres-Martínez, Cadena-Roa, & Métailler, 1984; Danielssen & Hjertnes, 1993; Regost et al., 2001) and on the development of tools for the identification of turbot with respect to other fish species (Etienne et al., 2000; Prost, Serot, & Demaimay, 1998). However, research concerning the quality changes that might occur during *post-mortem* storage only includes high pressure (Chevalier, LeBail, & Ghoul, 2001) and thermal (Madeira & Penfield, 1985) treatment, such that – to date – the mechanisms of damage taking place in farmed turbot during chilled storage remain relatively unknown.

In the light of this situation, in the present work, we were prompted to investigate the biochemical changes involved in the loss of quality undergone by farmed turbot during chilled storage. To this end, the changes in the most relevant biochemical components – lipids, proteins and nucleotides – were evaluated during a long storage period (29 days) in ice and complemented by sensory analysis.

2. Materials and methods

2.1. Raw material, sampling and processing

Two-year old farmed turbot (*P. maxima*) specimens were obtained from Stolt Sea Farm, S.A. (Carnota, Galicia, Spain). Fish specimens were sacrificed in a water-ice mixture and then kept in ice for 10 h until they arrived at our laboratory. The length of the fish was in the 39–46 cm range, while the width was in the 29–35 cm range; the weight range was 1.600–1.900 g. All the fish specimens were stored on ice in an isothermal room at 4 °C. Samples were taken for analysis on days 0, 2, 5, 9, 14, 19, 22, 26 and 29. Three different batches were laid down and studied separately along the whole experiment. Once whole fish had been subjected to sensory analysis, the white muscle was separated and homogenised to obtain extracts for analyses.

2.2. Sensory analysis

This was conducted by a taste panel consisting of five experienced judges, based on traditional guidelines concerning fresh and chilled fish (DOCE, 1989). Four categories were ranked: highest quality (E), good quality (A), fair quality (B) and unacceptable quality (C). Sensory assessment of the fish included the following parameters: skin, external odour, gills, consistency and flesh odour.

2.3. Chemical composition

The water content was determined by weight difference of the homogenised muscle (1–2 g) before and after heating at 105 °C for 24 h. The results were calculated as g water kg^{-1} muscle.

The lipid fraction was extracted by the Bligh and Dyer (1959) method. Quantification results was calculated as g lipids kg^{-1} wet muscle.

Two different protein extraction procedures were considered in this work. Preparation of urea-soluble protein extracts was carried out in extraction buffer A [8 M urea + 4% (w/v) CHAPS (3,3-chloramidopropyl-dimethylammonio-1-propanesulphate) + 40 mM Tris]. Sarcoplasmic protein extracts were prepared in a low-ionic-strength buffer – extraction buffer B [10 mM Tris–HCl, pH 7.2, +50 mM pentamethyl sulphonic acid]. In both cases, 500 mg of muscle were homogenised for 60 s in 4 ml of the required buffer solution, as previously described (Piñeiro et al., 1999). Then, all extracts were centrifuged at 12,500 rpm for 15 min, in a JA20.1 rotor (J221-M centrifuge, Beckman-Coulter, London, UK) at 20 °C, and the supernatants were recovered. Extracts prepared in buffer A were kept for 30 min at room temperature before spinning. All extracts were maintained at −80 °C until analysis.

Protein concentrations in the extracts were determined as follows: extracts prepared in buffer A were subjected to the PlusOne 2-D Quant kit (Amersham Biosciences, Uppsala, Sweden) while the protein concentration in extracts prepared in buffer B were determined by means of the protein microassay method (Bio-Rad Laboratories Inc., Hercules, CA, USA). In both cases, a standard curve, constructed for bovine serum albumin, was used as reference and results were expressed as g kg^{-1} muscle.

2.4. Nucleotide analysis

Nucleotide extracts were prepared according to the method of Ryder (1985) and stored at −30 °C until analysis.

Nucleotide analysis was performed by HPLC, using a Beckman device provided with the programmable solvent module 126 (Beckman), and the scanning detector module 167 (Beckman) connected to the System Gold software, version 8.1 (Beckman). Separations were achieved on a reverse-phase Spherisorb ODS-2 C_{18} 250 × 4.60 mm column (Waters, Milford, MA), with an internal particle diameter of 5 μm. The composition of the mobile phase was as follows: solvent A was composed of 0.04 M KH_2PO_4 + 0.006 M K_2HPO_4, pH 7; solvent B was acetonitrile. Solvents were filtered through a 0.45 μm aqueous filter before use. Separations were carried out using a continuous gradient elution with solvent A and solvent B. Table 1 summarises the tech-