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The influence of starvation time prior to slaughter on the quality of commercial-sized gilthead seabream (*Sparus aurata*) during ice storage

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ABSTRACT

Farmed commercial-sized gilthead seabream were subjected to different periods of starvation (24, 48 and 72 h) before being slaughtered in order to study the effect this had on *post mortem* quality and shelf-life. Once slaughtered (by immersion in a 1:3 ice water mixture), the animals were stored on ice at 4 °C for 0, 7, 14, and 21 days. In this manner, 12 groups were formed, each subjected to a different combination of starvation periods and storage times. At each point during the sampling, physical–chemical (pH, TBA, TVBN, color and texture), microbiological and sensory analyses were performed to determine the spoilage that had occurred in the fish. Higher pH values were found in animals that were starved for 24 or 48 h, than in those starved for 72 h. These values were the highest on days 14 and 21. Variations in TBA were not significant in any of the treatments. The TVBN increased with the number of days stored on ice. Color variations were most significant on the dorsal parts of the fish, with a discoloration occurring as the days went by in ice storage. The texture analysis revealed a certain softening of the flesh with time spent in storage, as well as a reduction in cohesiveness as the starvation period was prolonged. The microbiological analysis (total aerobic and *Pseudomonas* counts) and the quality index showed very significantly the deterioration in gilthead seabream as the on-ice storage time and the starvation time increased. The shelf-life was estimated to be 16 days for seabream starved for 24 h, 15 days for those starved for 48 h and 14 days for those starved for 72 h.

1. Introduction

The demand for fish and other aquaculture products has experienced a worldwide increase that has been especially significant in Europe and other regions. A growing percentage of human fish consumption is supported by aquaculture (FAO, 2006). Currently, aquaculture in the Mediterranean is practically centered on seabream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) farming. With a high commercial value, gilthead seabream is already well established on the market, having reached very high production levels in only one decade. Consequently, intensive and semi-intensive gilthead seabream production has increased significantly over the past years (Flos et al., 2002; Huidobro and Tejada, 2004). The increase in demand for this fish from the Mediterranean coast to Northern European countries has resulted in a more competitive market and very demanding consumers. As a result, there is now a need to obtain high-quality aquaculture products in order to further develop this sector. It is, therefore, of considerable interest to the farming industry, retailers and consumers alike to investigate the changes in cultured seabream quality that occur during its handling, distribution and storage on ice (Alasalvar et al., 2001).

Starving the fish for a few days before slaughter is a common practice in aquaculture. Flos et al. (2002), Huidobro et al. (2001), and Huidobro and Tejada (2004) each mention periods of feeding interruption lasting 24 and 48 h in order to empty the gastrointestinal tracts of the fish. More recently, in a questionnaire sent to aquaculturists, Ferreira Pinto et al. (2007) observed that 1 day was considered to be the minimum feeding interruption period, with 8 days being the maximum. Reasons for extending this period beyond 48 h included variations in the market price for the fish and the time needed to empty the fishpond. During feeding periods, the digestive tract of the fish contains many bacteria that produce digestive enzymes capable of causing intense *post mortem* autolysis, resulting in strong odors and flavors, especially in the abdominal area (Huss, 1995). By reducing the amount of faeces in the intestines, spoilage is delayed, and digestive enzyme activity is reduced after *rigor mortis* has occurred. If further processing steps are considered, e.g. filleting and freezing, feeding interruption may be a determinant of the product shelf-life (Huidobro and Tejada, 2004).

The quality of fresh fish is a major concern to the industry and consumers (Chytiri et al., 2004). Fish is an extremely perishable food commodity. Freshness is one of the most important attributes when evaluating the quality of the fish. The freshness and quality of the end product are dependent on different biological and processing factors that influence the degree of various physical, chemical, biochemical

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and microbiological changes that occur *post mortem* (Huss, 1995), and which result in a progressive loss of food characteristics in terms of taste and the general perception of quality (Olafsdottir et al., 1997). Both the time elapsed since slaughter and the storage temperature are key factors in determining the ultimate quality of the product. In fact, fish spoilage depends mostly on temperature, which controls to a large extent the bacterial and autolytic breakdown. Moreover, the rate of spoilage depends on several factors, such as the fish species and the amount of food found in the intestines (Macagnano et al., 2005). An estimate of freshness can be obtained by defining criteria related to changes in sensory attributes, such as appearance, odor, color and texture, which can be measured and quantified by sensory or instrumental methods (Olafsdottir et al., 2004). Microbiological, physical–chemical and sensory methods are used to determine these parameters, albeit sensory analysis is the most reliable method to determine fish freshness. For this reason, all the physical–chemical and microbiological methods must be combined and demonstrate a strong correlation with this type of analysis.

Therefore, the objective of this project was to study the influence that the starvation period prior to slaughter (24, 48 or 72 h) has on the quality and safety parameters (physical–chemical, microbiological and sensory) for commercial-sized gilthead seabream throughout their storage on ice.

2. Materials and methods

2.1. Animals and housing

Gilthead seabream, with an initial average weight of 436.8±87.3 g, were obtained from Culmarex S.A. farm (Águilas, Murcia, Spain) and kept at the IMIDA aquaculture facilities (San Pedro del Pinatar, Murcia, Spain). The fish were divided among six 850-l cylindrical–conical tanks, which were supplied with running seawater (salinity: 37 g/l; NO^{-2}: <0.1 mg/l; NO^{-3}: <0.1 mg/l; NH_3: <0.5 mg/l; and pH: 7.7). The tanks were part of a recirculation system equipped with biological filtration, an ultraviolet lamp and a thermostat to control the experimental temperature. The water flow was constantly regulated to maintain dissolved oxygen at 70% of the saturation level. Animals were subjected to natural photoperiod (37°50′N, 0°46′W) conditions at a constant temperature (21.1±2.1 °C), and were fed a commercially available seabream diet (44% protein, 22% fat, 8.5% ash and 1.3% fiber) two times a day to satiety.

2.2. Experimental design

The animals needed a period of 4 weeks to adapt to the conditions found at the installations. They began to eat regularly, reaching an adequate ingestion rate for their body weight at 2 weeks, and with starvation starting 2 weeks later. During the starvation period, three samples were taken (at 24, 48 and 72 h following the start of the starvation), obtaining three groups of animals that had each been subjected to a different type of starvation. They were killed by hypothermia, using a mix of water and ice (1:3). A total of 144 animals were slaughtered, 48 for each starvation period.

Each starvation group was divided into four groups of 12 animals each and stored for 0, 7, 14 and 21 days, respectively. Day zero was considered to be the real period of time of 24 h following slaughter. There were no external variation factors. They were kept in refrigeration at 4±1 °C, placed in polystyrene boxes with outlets for water drainage and covered with flaked ice inside a plastic bag. The fish were positioned on their sides, with one side of their bodies in contact with the box and the other with the ice. Both the refrigeration temperature and the ice/fish ratio (1:1) were maintained constant throughout the experiment.

Eight fish from each group were used for texture evaluations, and the remaining four for the rest of the analyses. Both the sensory evaluation and the color measurement were estimated using whole fish. They were later filleted and homogenized, using the fillets from the right side for the microbiological analysis and from the left side for the physical–chemical analysis.

2.3. Analytical determinations

2.3.1. Chemical analysis

Five grams of each sample were blended with 15 ml distilled water, and the pH value of the fish homogenate was measured using a digital pH-meter (ORION, Beverly, MA, USA), standardized at pH 4.01 and 7.00. Thiobarbituric acid (TBA, mg malonaldehyde/kg fish flesh) was determined according to the method used by Botsoglou et al. (1994), and total volatile basic nitrogen analysis (TVBN, mg N/100 g fish flesh) was performed according to the EU reference (EEC, 1995). All analyses were performed in duplicate.

2.3.2. Physical analysis

A Minolta Chroma Meter CR400 (Minolta, Osaka, Japan) was used for color measurements. Colors were expressed as CIELab coordinates. In this system, L^* denotes lightness on a 0–100 scale of black to white; a^*, (+) red or (−) green; b^*, (+) yellow or (−) blue. Color intensity is expressed by chroma value, and hue is the name of a color as it is found in its pure state on the spectrum. Both values were calculated using the formulae: $C^*_{ab}=(a^{*2}+b^{*2})^{1/2}$ and $H^o_{ab}=\arctan(b^*/a^*)$. Eight color measurements (four per body side) were performed on each individual, as described by Pavlidis et al. (2006); two on the dorsal skin area, (i) at the positions where the vertical line to the longitudinal body axis passes through the anterior margin of the dorsal fin (D1) and (ii) through the anus (D2), and two at the ventral skin area, (i) below the pelvic fin (V1) and (ii) at the position where the vertical line to the longitudinal body axis passes through the anus, crossing the parallel line to the longitudinal body axis as it passes through the ventral margin of the caudal peduncle (V2) (Fig. 1).

The texture profile analysis (TPA) was conducted using a texture analyzer QTS-25 (CNS Farnell, Borehamwood, Hertfordshire, England) equipped with a 25 kg load cell and Texture Pro V. 2.1 software. The samples were compressed perpendicular to the fish body, using a 20 mm-diameter cylindrical probe. The testing conditions were: two consecutive cycles of 25% compression; cross-head movement at a constant speed of 50 mm/min, and a trigger point of 0.05 N. Texture variables (hardness, gumminess, adhesiveness, cohesiveness, chewiness and springiness) were calculated as described by Bourne (1978). Measurements were made on the left side of the fish, and were taken from the central part of the body. After cutting the pectoral fin, an approximate distance of 3 cm was measured from its origin, in the middle of the body.

2.3.3. Microbiological analysis

For microbiological counts, 10 g of sample were mixed with 90 ml of 0.1% peptone–water (Oxoid code CM 9) and homogenized with a

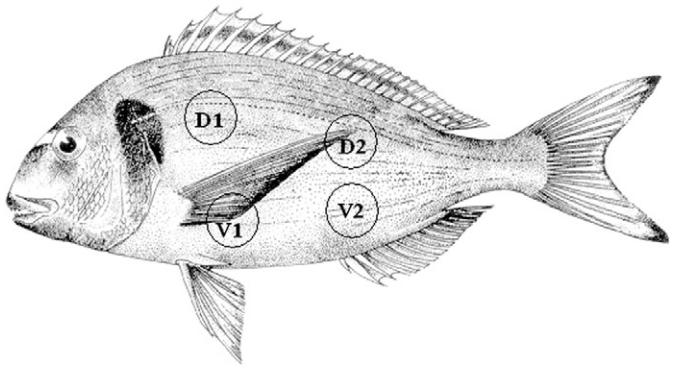

Fig. 1. Representation of the points on the seabream where color was measured.