TESIS DOCTORAL

EFECTO DE LA DIETA SOBRE LA MORFOLOGÍA MUSCULAR Y LA CALIDAD Y VIDA ÚTIL DEL FILETE EN EL BESUGO (*Pagellus bogaraveo*)

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INFORMA,

De que la Comisión Académica del Programa de Doctorado, en su sesión de fecha ..... tomó el acuerdo de dar el consentimiento para su tramitación, a la tesis doctoral titulada "EFECTO DE LA DIETA SOBRE LA MORFOLOGÍA MUSCULAR Y LA CALIDAD Y VIDA ÚTIL DEL FILETE EN EL BESUGO (*Pagellus bogaraveo*)" presentada por la doctoranda D/Dª Laura Rincón Martínez y dirigida por el Doctor Rafael Ginés Ruiz y el Doctor Pedro Luis Castro Alonso.

Y para que así conste, y a efectos de lo previsto en el Artº 11 del Reglamento de Estudios de Doctorado (BOULPGC 04/03/2019) de la Universidad de Las Palmas de Gran Canaria, firmo la presente en Las Palmas de Gran Canaria, a...de.....de dos mil.....

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Título de la Tesis: EFECTO DE LA DIETA SOBRE LA MORFOLOGÍA MUSCULAR Y LA CALIDAD Y VIDA ÚTIL DEL FILETE EN EL BESUGO (Pagellus bogaraveo)

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CAPÍTULO 1. INTRODUCCIÓN

La acuicultura marina en el sur de Europa se ha basado fundamentalmente en dos especies, la dorada (*Sparus aurata)* y la lubina (*Dicentrarchus labrax*). La expansión del sector junto con la disminución de las capturas de otras especies habituales en el mercado, lleva hacia el creciente interés en la diversificación que desde la acuicultura pueda satisfacer las crecientes demandas de los consumidores. En este contexto, una especie como el besugo (*Pagellus bogaraveo*), con un elevado valor comercial debido a sus características organolépticas, ha llegado a ser una alternativa razonable de cara a ser producido de forma controlada (Peleteiro *et al*., 2000). A ello se añade que su sobreexplotación prácticamente ha colapsado las pesquerías tradicionales (Lorance, 2011; Pinho *et al.*, 2014), con unas capturas que han disminuido desde las 8.910 toneladas en 1980 hasta las 1.493 en 2018 (FAO, 2020).

Es conocido que la producción acuícola controlada promueve variaciones en la composición bioquímica de los peces que un claro efecto sobre su calidad (Izquierdo *et al*., 2003). Así, el tipo y cantidad de ingredientes con los que se elaboran las dietas, junto con el constante aporte de alimento que la propia intensificación productiva obliga, deviene en unos mayores depósitos de grasa tal cual ya ha sido claramente constatado en especies como la dorada (Grigorakis *et al*., 2002; Castro *et al.*, 2010), lubina (Alasalvar *et al*., 2002; Fuentes *et al.*, 2010), halibut (*Hippoglossus hippoglossus*) (Olsson *et al.*, 2003) o rodaballo (*Scophthalmus maximus*) (Martinez *et al*., 2010), cuando son comparados con individuos de esas misma especies procedentes de la pesca extractiva.

Esos ingredientes de la dieta previamente mencionados, al menos los que se venían utilizando de manera tradicional, léase harinas y aceites de pescado, están sometidos a un incremento de su demanda a nivel global, lo que ha conllevado un importante incremento en el coste de las dietas destinadas a los peces de crianza (Tacon y Metian, 2008; Castro *et al*., 2015). Precisamente es por esto por lo que la producción de besugo puede representar una interesante oportunidad, ya que, aun siendo una especie carnívora, mantiene unos razonables rendimientos productivos a pesar de llegar a reducir de manera importante las inclusiones de aceite de pescado en su dieta (Figueiredo-Silva *et al*., 2009). Esta opción pasa, en cualquier caso, por un aumento de la energía disponible en la dieta a través de la inclusión de carbohidratos, únicamente llegando a ser efectiva cuando la fuente de proteína proviene mayoritariamente de la harina de pescado (Valente *et al.*, 2010).

Los estudios nutricionales llevados a cabo en de besugo han estado centrados en los efectos de los distintos niveles de alimentación sobre la respuesta de crecimiento,

composición bioquímica y balances energéticos (Peleteiro *et al*., 2000; Olmedo *et al.,* 2000; Linares *et al*., 2001, Silva *et al*., 2006; De Almeida Ozório *et al*., 2009). Y así, se ha concluido que el besugo es menos eficiente que otros espáridos en referencia a la conversión del alimento, ya que requiere un mayor contenido proteico en la dieta (Silva *et al*., 2006). Las dietas comerciales para esta especie alcanzan un mínimo de 45-50% de contenido en proteína de cara a garantizar la mejor tasa de crecimiento, pero menos de la mitad de esta proteína se corresponde con harina de pescado. Debido a esto, el potencial de crecimiento podría verse disminuido tras la inclusión de esas otras fuentes de proteína (Figueiredo-Silva *et al*., 2010), las cuales van a tener origen vegetal.

Es evidente que desde los fabricantes de pienso se persiga una reducción de los costes a través no sólo de la sustitución de las harinas de pescado por harinas vegetales, sino también de los aceites de pescado, con la clara reducción de aporte de energía que ello conlleva. Esta pérdida energética podría ser contrabalanceada a través del incremento de carbohidratos, pero sólo puede llegar a ser efectiva cuando la fuente de proteína es la harina de pescado (Valente *et al*., 2010). En definitiva, la posible ventaja económica aparejada a la reducción de los aportes de aceite de pescado en las dietas para besugo, se ve mermada por la limitada sustitución de las harinas de pescado por harinas vegetales, ya que las primeras son necesarias de cara a mejorar el uso de los carbohidratos incluidos en la dieta (Figueiredo-Silva *et al*., 2009; Valente *et al*., 2010).

Si bien los efectos sobre la composición bioquímica del músculo se han evaluado sobre juveniles de besugo (Silva *et al*., 2006; Parmigiano *et al*., 2007), los cambios en las dietas deben culminarse en ejemplares similares a los que se consideran de talla comercial, almacenados en hielo y presentados en el mercado para que puedan ser tenidos en cuenta para la decisión de compra por parte del consumidor. Bajo todas estas condiciones, las características del músculo, la valoración de los atributos sensoriales, o el color de la piel, son esenciales de cara a determinar la aceptabilidad del producto.

Atendiendo a la calidad del pescado, la textura es uno de los parámetros más importantes, no sólo para los productores sino también para los consumidores (Hyldig y Nielsen, 2001), máxime teniendo en cuenta que los cambios en la firmeza que acontecen a lo largo de la vida útil del pescado a lo largo del periodo de conservación en hielo están estrechamente asociados con dicha percepción de calidad (Cheng *et al*., 2014). En el pescado de crianza, el mayor contenido en lípidos del músculo y su distribución, influyen en las propiedades de textura del filete (Lie, 2001). Suárez *et al.* (2010) concluyeron que, tras una restricción alimenticia, se mejoraba la textura del músculo debido al descenso de los depósitos lipídicos en los componentes estructurales

del músculo. Sin embargo, al comparar los efectos del aporte de dietas isoenergéticas, las variaciones debidas a las posibles modificaciones de la proteína o del contenido en lípidos, no se vieron afectadas las características de textura, excepto aquellas promovidas por los cambios en el área de las fibras musculares (García de la Serrana *et al.*, 2013).

Entre el 40 y el 60% de la masa corporal de los peces lo constituye el músculo esquelético (de Almeida *et al*., 2010), correspondiéndose además con la pare consumible. Su estructura en los peces de crianza ha sido comúnmente asociada a una menor firmeza que la presentada por los de pesca extractiva (Periago *et al.*, 2005). Factores de especial relevancia durante la crianza tales como la composición de la dieta y el régimen alimenticio (Kiessling *et al*., 1991), el ejercicio (Totland *et al*., 1987) y la temperatura (Ayala *et al*., 2001; López-Albors *et al*., 2003; Johnston *et al*., 2003)., provocan variaciones en la evolución y desarrollo de las fibras musculares a lo largo del crecimiento del pez, tanto en su distribución como en su tamaño. Este efecto hay que destacarlo enmarcado en dos procesos fundamentales, la hiperplasia mediante el reclutamiento de nuevas fibras y la hipertrofia a través del crecimiento de aquellas fibras previamente formadas (Egginton and Johnston, 1982), todo ello como resultado del balance entre síntesis y degradación de la proteína (Alami-Durante *et al*., 2010a). De acuerdo con esto, los estudios histológicos atendiendo al reclutamiento de fibras, así como a su morfología y distribución, son un importante instrumento para determinar las propiedades de textura de la especie en estudio.

Las modificaciones achacables a las condiciones de crianza referidas a los cambios en las dietas pueden ser registradas mediante protocolos de valoración sensorial, principalmente para aquellos atributos relacionados con la textura de la carne Olsson *et al*., 2003; Sveinsdóttir *et al*., 2009) así como del sabor (Grigorakis *et al*., 2003). Mediante técnicas instrumentales, Periago *et al*. (2005) en lubina, y Johnston *et al*. (2006) en salmón Atlántico (*Salmo salar*), determinaron que los filetes crudos de individuos provenientes de la pesca extractiva presentaban una textura más firme que los de crianza. Todavía más, debido a los cambios en la estructura del filete debido a los propios procesos térmicos que conlleva el cocinado, ligeros valores casi imperceptibles en el filete crudo pueden no llegar a ser detectados mediante el análisis sensorial (Castro *et al*., 2015; Hu *et al*., 2013). Por su parte, las variaciones en los sabores se explicarían por las diferencias en la composición bioquímica del músculo (Grigorakis *et al*., 2007) y el perfil de ácidos grasos (Izquierdo *et al*., 2005; Montero *et al*., 2005).

El análisis sensorial descriptivo es el método más adecuado para obtener resultados cuantitativos que caractericen la calidad sensorial, pero puede variar considerablemente en los productos acuícolas dependiendo de la especie con la que se trabaje (Hyldig, 2007). Al trabajar con paneles de evaluadores entrenados, es necesario definir una nomenclatura correcta para describir los atributos a evaluar (Lawless y Civille, 2013), logrado con ello una útil herramienta para establecer posibles diferencias entre los lotes sometidos a diferentes factores de producción, en este caso la dieta. Así, la implementación de un perfil sensorial podría ayudar al sector tanto a evaluar sus productos (Warm *et al*., 2000) como a establecer un control de calidad durante el proceso de producción (Sveinsdóttir *et al*., 2010). En definitiva, de cara a conocer la aceptación por parte de los consumidores de peces de diferentes orígenes, se hace obligado identificar y definir las propiedades sensoriales (Drake *et al*., 2006). Factores como el origen, ya sea de pesca extractiva o de crianza, influye en dichas características sensoriales (Green-Petersen *et al*., 2006).

El color en el pescado, más que un atributo de apariencia, ejerce una clara influencia en la aceptabilidad del producto, así como de su valor en el mercado (Kalinowski *et al*., 2011). Si bien en los salmónidos el color del filete es un factor determinante de su consideración de calidad (Sigurgisladotir *et al*., 1997), en el caso de los espáridos los cambios del color no son tan manifiestos, aunque si algunas variaciones están relacionadas con el origen (Grigorakis *et al*., 2003), el sistema de crianza (Valente *et al*., 2011) o la dieta (Menoyo *et al*., 2004), llegando en estos casos a promover ligeras variaciones en el color del filete.

El besugo tiene un característico color de la piel que como tal permite establecer diferencias en la valoración de la frescura mediante la metodología del QIM (Sant'Ana *et al.*, 2011). Pero los peces con un color de la piel identificativo y particular de la especie, pueden oscurecer y virar hacia tonos grisáceos bajo las condiciones de la propia crianza (Kalinowski *et al*., 2005). Conocer la tendencia y magnitud de estos cambios de color puede apoyar ajustes en la dieta que garanticen la correcta proporción de pigmentos rojos y amarillos en la piel y por ello conseguir criar peces con una apariencia global más adecuada a las expectativas de los consumidores (García-Romero *et al*., 2014). Más aun, el patrón de pigmentación de la piel puede ser considerado como un índice de bienestar animal en los peces de crianza, especialmente importante en el caso de especies con un color de la piel rojo-rosado que va a oscurecerse bajo las condiciones de cría intensiva (Pavlidis *et al*., 2006).

OBJETIVOS

A la vista de todo lo expuesto, el objetivo general del presente trabajo sería la caracterización de la calidad del besugo de crianza, atendiendo especialmente a las dietas comerciales que en la actualidad se están utilizando, pero sin olvidar las tendencias a que se ven forzados los fabricantes de piensos por un lado en relación al coste de las materias primas, y por otro, no menos importante, a las propias características de un pescado magro.

Para abordar todo esto en la secuencia de los diferentes estudios realizados, se plantean los siguientes objetivos parciales:

1.- Determinar las diferencias organolépticas entre besugos de pesca y de crianza, así como de su respectivo valor nutricional, textura y color de la piel y de la carne.

2.- Evaluar los cambios sensoriales y físico-químicos durante el periodo de vida útil tras el sacrificio, con especial referencia a la posible modificación del valor nutricional, en función del origen y tipo de dieta.

3.- Valorar los cambios en la estructura muscular en función del origen y tipo de dieta y su repercusión en la evolución de la textura durante el periodo de vida útil tras el sacrificio.

CAPÍTULO 2. Differences in proximal and fatty acid profiles, sensory characteristics, texture, colour and muscle cellularity between wild and farmed blackspot seabream (*Pagellus bogaraveo*)

Aquaculture, 451, 195-204

Abstract

In order to determine differences between wild and farmed blackspot seabream, a promising finfish species for aquaculture, some parameters affecting quality were evaluated, such as proximal and fatty acid profiles, texture, skin and muscle colour and muscle cellularity, along with an extensive sensory assessment. Proximal composition showed a higher fat proportion in the farmed group whilst higher collagen content was found in the wild group. The fatty acid profile of the farmed group showed important values of EPA and total *n*-3 HUFA. Sensory evaluation registered changes in all tested features, especially seafood attributes linked to wild fish and fish oil attributes associated with the farmed group. Texture studies were focused on whole fish and on fillets, both raw and cooked. Raw wild fish fillet showed higher values for hardness and fracturability than farmed fillet. Cooked farm fish fillet rendered higher springiness values than those observed in the wild group. Significant variations were determined in colour studies with a higher lightness (L*) and redness (a*) on the skin of the wild fish and a higher Hue than farmed fish. The analysis of fibre type showed that red muscle area was extensive in farmed fish in cranial, medial and caudal areas. Additionally, the fibre morphology of the red muscle of wild fish showed a greater amount of smaller fibres than that observed in farmed specimens in the three studied areas. The fibres with smaller girth on white muscle were only found in the cranial section of the wild fish.

2.1. Introduction

In Southern Europe, marine aquaculture has been focused on the production of limited species like the gilthead seabream (*Sparus aurata)* and the European seabass (*Dicentrarchus labrax*). With the expansion of the aquaculture sector along with dwindling stocks of traditional species, there has been increased interest to develop new finfish species. Because of its high commercial value due to its firm and flavourful flesh, blackspot seabream (*Pagellus bogaraveo*) farming has become a market alternative to the overexploitation and collapse of traditional fisheries (Lorance, 2011). Captures have diminished from 5519 tonnes in 1982 to 1082 tonnes in 2012, while farmed production has remained relatively steady at around two hundred metric tonnes per year (FAO, 2014). Fish aquaculture production results in compositional variations that affect flesh quality (Izquierdo *et al*., 2003). Farmed fish diets and the constant supply of food modify the proximal and fatty acid profile and result in the deposit of large amounts of lipid reserves, previously shown in different farmed species compared to their wild

counterparts, such as the gilthead seabream (Grigorakis *et al*., 2002), European seabass (Alasavar *et al*., 2002a; Fuentes *et al*., 2010), Atlantic halibut (Olsson *et al*., 2003) or turbot (Martinez *et al*., 2010).

These variations attributable to rearing conditions can be registered by sensory evaluation, mainly in attributes related to flesh texture (Olsson *et al*., 2003; Sveinsdóttir *et al*., 2009) and taste (Grigorakis *et al*., 2003). By instrumental means, Periago *et al*. (2005) in seabass and Johnston *et al*. (2006) in salmon (*Salmo salar*) found that the raw fillet of wild fish has a firmer texture than that of farmed fish. To add further to this profile, it has been reported that fillet structure is modified during the thermal process of cooking and so differences in texture values imperceptible in raw fillet studies could now be detected by sensorial assessment (Castro *et al*., in press; Hu *et al*., 2013). Taste variations could be explained by the different chemical muscle composition (Grigorakis *et al*., 2007), and fatty acid profile (Izquierdo *et al*., 2005; Montero *et al*., 2005).

Descriptive sensory analysis, such as profiling, is the most resourceful method capable of providing quantitative data to give a characterization of sensory quality, but it can vary considerably in seafood depending on species (Hyldig, 2007). Sensory analysts, working with trained descriptive panels, need well defined nomenclature to consistently and correctly describe products of interest (Lawless and Civille, 2013), providing a tool for understanding the differences among products. Implementation of the sensory profile will help the fish industry to compare products (Warm *et al*., 2000) or with quality control during product development (Sveinsdóttir *et al*., 2010). In order to understand consumer acceptance of fish from different sources, it is necessary to identify and define sensory properties (Drake *et al*., 2006). Factors such as the fish origin, farmed or wild, influence the sensory properties of the products (Green-Petersen *et al*., 2006).

Colour in fish is more than an appearance attribute as it has a significant effect on product acceptability and market value (Kalinowski *et al*., 2011). In salmonids the flesh colour is considered a factor to determine product quality (Sigurgisladotir *et al*., 1997). In sparids colour changes are not so prominent but some variations related to origin (Grigorakis *et al*., 2003), rearing system (Valente *et al*., 2011) or diet (Menoyo *et al*., 2004) could promote slight variations in flesh colour. The blackspot seabream has a distinctive red-pink skin colour, a feature that has been included in the QIM since it is so characteristic (Sant'Ana *et al.*, 2011). Fish species with an identified skin colour could turn dark grey under captivity (Kalinowski *et al*., 2005). The acquisition of knowledge on the tendency and magnitude of the skin colour changes produced under farming conditions and adjustments of the diet that leads to the right proportion of red and yellow pigments in

the skin of farmed fish could be useful to attain an adequate overall colour appearance (García-Romero *et al*., 2014).

Skeletal muscle forms 40-60% of the body mass in most of fish (de Almeida *et al*., 2010) and is the main edible part of the fish. The skeletal muscle composition during the rearing period is affected by hypertrophy and hyperplasia phenomena until muscle fibres reach a functional maximum diameter (Egginton and Johnston, 1982). The number and girth of muscle fibres can be affected by rearing conditions such as diet (Kiessling *et al*., 1991), exercise (Totland *et al*., 1987) and temperature (Ayala *et al*., 2001; López-Albors *et al*., 2003; Johnston *et al*., 2003). Muscle fibre distribution and composition variations, and their connections with sensory values associated to farming or wild lifestyles have attracted interest in fish species (Periago *et al*., 2005; Ayala *et al.,* 2001). Blackspot seabream studies have been focused on nutrition (Silva *et al*., 2006, 2009; Figueiredo-Silva *et al*., 2010) and muscle fibre evolution (Silva *et al*., 2008, 2010, 2011) up to juvenile age.

Consumers give a moderate importance to the method of obtaining the fish, be it capture or farming (Claret *et al*., 2012). However, wild fish are considered to be of a better overall quality than farmed fish, mainly in relation to sensory characteristics like better taste and a firmer texture, as well as more nutritious (Claret *et al*., 2014). Accordingly, taking into account that the improvement of sensory and compositional knowledge becomes a tool to determine those farming conditions that match consumer's preferences, the present work focuses on quality differences comparing commercial size wild and farmed blackspot seabreams, together with the development of the species profile from a sensory assessment.

2.2. Materials and methods

2.2.1. Experimental fish

Farmed blackspot seabreams (*Pagellus bogaraveo*) were reared in net cages at Polygon B of Bueu Bay (Pontevedra, Spain). During the growing period, fish were fed a commercial diet (Bes-Power, Sparos, Faro, Portugal; Table 1). Once this period was complete, fish with a commercial weight of around 527.1±45.6 g were slaughtered by immersion in ice cold water (fish:ice rate 2:1). Samples of whole ungutted fish with flaked ice were packed into polystyrene boxes and shipped to the different associated research Institutes: IRTA (Gerona, Spain), IMIDA (Murcia, Spain) and IUSA (Gran Canaria,

Spain). Wild blackspot seabreams, with an average weight of 551.2±46.4 g., were obtained from Vigo Fish Market (Pontevedra, Spain) when arriving at the port and packed and shipped as described above. Experimental fish were distributed in the following way: sixteen wild and sixteen farmed seabreams were sent to each panel (IRTA, IMIDA and IUSA) for sensory evaluation. Additionally, thirty wild and thirty farmed fish were sent to the IUSA laboratories for chemical, physical and histological determination. These were divided in the following way: six wild and six farmed whole fish were analyzed for skin colour and whole fish texture, evaluating the left side of the fish. After that, fish were filleted and the right side fillets (thirty) were devoted to proximal and fatty acid analysis. The remaining 24 left fillets were devoted to flesh colour, fillet texture (raw and cooked) and histology studies (six fillets per study).

Table 1. Ingredients (g/kg) and chemical composition (% dry basis) of the diet.

Ingredient		Composition	
Fishmeal	270	Crude protein	46 %
Soybean meal	200	Crude lipids	24 %
Corn gluten	180		
Wheat gluten	90		
Bean meal	40		
Pea meal	10		
Fish oil	170		
Soybean oil	30		
Premix	10		

2.2.2. Sensory evaluation

2.2.2.1. Testing panels

Sensory panels were set in the institutes (IRTA, IMIDA and IUSA) in order to establish the blackspot seabream Quantitative Descriptive Analysis (QDA). Panels were composed of twelve judges specifically selected for their previous experience in fish

quality boards, trained according to ISO guidelines (ISO, 8586-2:2008). To determine the intensity of tested attributes, several species easily available in local markets were selected: hake (*Merluccius merlucius*), Atlantic salmon, halibut (*Hippoglossus hippoglossus*), sole (*Solea solea*), red banded seabream (*Pagrus auriga*), angler (*Lophius piscetorius*), mullet (*Mugil cephalus*), striped catfish (*Pangasianodon hypophthalmus*) and swordfish (*Xiphias gladius*). Eighteen different attributes of odor, appearance, texture, flavor and residual aftertaste were included in the description of the blackspot seabream profile (Table 2). Once the QDA was established, four training sessions (two a week) with wild and farmed blackspot seabreams were carried out.

2.2.2.2. Sample preparations

Sixteen wild and sixteen farmed blackspot seabreams were assessed per panel. After filleting, three rectangular pieces (3 x 4 cm) were processed from each fish fillet. Pieces with skin were cooked in lidded aluminum boxes in an air heated oven (Compact; Eurofred, Barcelona, Spain) at 115 °C for ten minutes.

2.2.2.3. Assessment

Sensory evaluation was carried out in a test room designed following ISO guidelines (ISO, 8589:2007). Each panelist assessed sets of four samples stored in a temperate maintainer (Clatronic International GmbH, Kempen, Germany). Assessments were recorded on a continuous scale with anchors presented on a computer screen and ranked from low (value 0) to high intensity (value 100) for each attribute.

2.2.3. Proximate composition and fatty acid profile

After reception, fish were weighed and their lengths were measured. Thirty wild and thirty farmed blackspot seabreams unskinned fillets (right side) were homogenised in batches of three and immediately devoted to proximal analysis by a FoodScan™ (FOSS, Hillerød, Denmark), (AOAC, 2007.04). Ash content was determined gravimetrically after combustion for 24 h at 450ºC (AOAC, 1995). Lipids from diets and fillets were extracted with a chloroform: methanol (2:1 v:v) mixture as described by Folch *et al*. (1957). Fatty acid methylesters were purified by adsorption chromatography on NH_2 Sep-pack

cartridges (Waters, S.A., Milford, Massachusetts), separated and quantified by gas-LC following the conditions described by Izquierdo *et al*. (1990), and identified by comparison to external standards.

Table 2. Sensory attributes for cooked blackspot seabream and attribute definitions.

Odour	
Global intensity	Global odour intensity
Seafood	Odour associated with seafood
Oily	Odour associated with fish oil
Appearance	
Whiteness	Intensity of white colour in the uncut steak
Shininess	Intensity of light in the uncut steak
Fillet integrity	Easiness to separate the miomers with the fork
Texture	
Firmness	Force required to deform the fillet between the tongue and palate
Juiciness	Amount of liquid released when the sample is chewed
Gumminess	Force required to separate the muscle miomers
Chewiness	Amount of chewing required before swallowing
Adhesiveness	Degree which the fillet clings to the teeth during chewing
Fatness	Degree of fat perception in the mouth during chewing
Flavour	
Global intensity	Global flavour intensity
Seafood	Flavour associated with seafood
Oily	Flavour associated with fish oil
Sweetness	Degree of taste slightly sweet
Roughness	Degree of taste slightly acid
Aftertaste	Degree of flavour persistence after swallowing

2.2.4. Texture and colour

Three texture analyses were carried out on six different fish from each of the wild and farmed groups: whole fish and raw and cooked fillets. Whole ungutted fish texture studies were developed on the left side, 1 cm approximately from the operculum and over lateral line. Each fish was compressed in two consecutive cycles to a depth of 7 mm with a plunger of 1.2 cm in diameter at a constant speed of 0.8 mm/s and 5 s between cycles. Tests attempted to simulate the pressure that might be applied by a person carrying out a sense evaluation (Ginés *et al*., 2002). For fillet texture studies, the skin was removed and three-square pieces (cranial, central and caudal, 2.5x2.5x1.5 cm) were collected from the left fillets, above the lateral line. The force-deformation curve was analyzed to determine four texture parameters (fracturability, hardness, springiness and adhesiveness). Compression plate and speed were 100mm Ø and 0.8mm/s, respectively. The deformation was 60% of the original thickness (Ginés *et al*., 2004). Cooked fillet samples were prepared as above and cooked in an air-heated oven (Compact; Eurofred, Barcelona, Spain) for 10 min at 115°C, packed in lidded aluminium boxes. In cooked fillet, deformation was 80% of the original length. Fracturability was not determined.

Colour was recorded from the skin and fillets of six different fish per group using a Colorimeter CR-400 (Konica Minolta, Osaka, Japan) following the system of CIE (1976). L* describing lightness (L*=0 for black, L*=100 for white), a* describing intensity in red (a*>0), b* describing intensity in yellow (b*>0), chroma (C_{ab}: $(a^{*2}+ b^{*2})^{1/2}$) and hue (H^*_{ab}:arctan (b*,a*). Skin colour was measured above the lateral line, 1 cm behind the operculum. Fillet colour was measured in a white muscle area. Determinations were made in triplicates rotating the illumination system 90º after each measurement.

2.2.5. Muscle fibre studies

Six fillets (left side) per group were cross sectioned at three different locations. Samples of 0.5 cm thickness from the cranial (the widest part), medial (middle of the fillet) and caudal (tail area) sections of the fillet were obtained. Samples were photographed and a portion from both red and white muscle was trimmed. Blocks 2mm thick were covered with tissue freezing medium (Tissue-Tek O.C.T., Sakura Finetek, Hatfield, PA), snap frozen over liquid nitrogen, wrapped in foil and stored at −80 °C until sectioning. Sections of 6-µm thickness were obtained in a cryostat (Slee, Mainz, Germany), fixed and stained with stained with haematoxylin and eosin (Pearse, 1985).

Proportion of muscle type, red and white, and fibre number among groups were examined with an image analysis package (Image-pro plus software, media cybernetics, Atlanta, GA) connected to a light photomicroscope (Olympus CX41, Tokyo, Japan). For muscle proportion, red muscle area was outlined and compared with white muscle area. To determine fibre number, three different microphotographs were randomly chosen per section (from both the red and white muscle areas) at a 40x objective magnification. The outlines of the muscle fibres were manually traced when necessary and the fibres counted, avoiding those incomplete in the image periphery.

2.2.6. Statistical analysis

Data were analyzed with IBM SPSS Statistics for Windows, Version 21.0 (Armonk, NY: IBM Corp.). For sensory evaluation a linear model with origin and panel as fixed factors was used to determine effects. Sensory data were standardized. Biochemical and colour measurements were compared using a one way model. Body weight was used as the co-variable for texture analysis in a linear model with origin as the fixed factor. In order to show the multivariate structure of the sensory evaluation, a principal component analysis (PCA) was conducted in the programme Unscrambler® v.10.0 (Camo, Oslo, Norway).

2.3. RESULTS

2.3.1. Sensory evaluation

Sensory results of the fish group (wild or farmed) are shown in Table 3 based on the developed blackspot seabream profile (Table 2). In the topic of odour, farmed fish fillets showed higher levels both in “intensity” and “oily” odour ($P < 0.000$). However, the “seafood” odour was perceived more intensely in wild samples ($P < 0.000$). The assessment of appearance attributes resulted in highest scores in fillets from wild fish. Thus, they were whiter, more shiny and needed more pressure to separate the miomers (“fillet integrity”) ($P < 0.000$). Regarding texture attributes included in the profile, “fatness” showed a clearly high intensity in farmed fish ($P < 0.000$). However, both “juiciness” sensation and perception of muscle structure when first chewing, determined by “gumminess”, obtained higher scores in wild fish. Meanwhile, the number of chews required to reduce the fillet to allow swallowing (“chewiness”) and the residue attached

to the teeth after that ("adhesiveness") were higher for fish of farmed origin. It is noticeable that "firmness" showed not differences between rearing methods.

Table 3. Average sensory scores for the attributes evaluated in farmed and wild blackspot seabream muscle and effect of factors origin and panel. Values are expressed as means ± SD. n=16

		Wild	Farmed	P-value		
				Origen	Panel	Org*Pan
Odour	Intensity	53.88 ± 13.80	57.88 ± 12.86	0.000	0.984	0.715
	Seafood	40.92 ± 18.56	33.38 ± 20.12	0.000	0.966	0.705
	Oily	26.35 ± 16.40	39.16 ± 18.86	0.000	0.944	0.908
Appearance	Whiteness	62.84 ± 15.99	58.08 ± 15.08	0.000	1.000	0.256
	Shininess	62.84 ± 14.59	55.17 ± 15.56	0.000	1.000	0.100
	Integrity	57.44 ± 15.58	51.80 ± 16.82	0.000	1.000	0.022
Texture	Firmness	51.78 ± 13.26	52.02 ± 14.35	0.793	1.000	0.013
	Juiciness	55.42 ± 13.86	50.81 ± 15.17	0.000	1.000	0.000
	Gumminess	45.64 ± 24.42	40.37 ± 19.53	0.012	0.999	0.045
	Chewiness	49.37 ± 16.39	52.63 ± 15.29	0.021	1.000	0.816
	Adhesiveness	43.17 ± 18.07	53.28 ± 18.16	0.000	0.999	0.026
	Fatness	31.68 ± 17.03	45.49 ± 19.92	0.000	0.904	0.359
Flavour	Intensity	49.99 ± 14.53	59.48 ± 13.89	0.000	0.999	0.698
	Seafood	40.82 ± 17.44	32.17 ± 19.62	0.000	0.931	0.499
	Oily	24.86 ± 15.37	44.10 ± 20.23	0.000	0.731	0.831
	Sweetness	39.53 ± 17.95	35.68 ± 17.22	0.008	0.912	0.914
	Roughness	35.37 ± 16.73	41.91 ± 17.68	0.000	0.970	0.236
Aftertaste	Persistence	45.07 ± 15.33	57.36 ± 14.17	0.000	0.559	0.494

Flavour attributes offered similar results to those relating to odour (Table 3), where “intensity” and “oily” had high scores in farmed and “seafood” was more noticeable in samples from the wild group ($P < 0.000$). It should be noted that the “oily” attribute obtained different scores for the two groups in odour and flavour, but panelists noticed a bigger difference between the groups for flavour. The other two attributes of flavour evaluated indicated a sweeter taste in the wild group ($P < 0.01$) and rougher in the farmed group ($P < 0.000$). The last profile attribute “aftertaste” was determined as more persistent in samples from the farmed group ($P < 0.000$).

It is worth mentioning that there is no effect of the panel on any attribute. There were very few attributes for which a statistically significant interaction between the panel and the origin of the fish was found, which reinforces the value of the sensorial interpretation of the panelists. These attributes are mainly related to texture such as “firmness”, “gumminess” and “adhesiveness”. This interaction, although significant, was ordinal in all cases.

The results of the multivariate structure are consistent with those obtained by the univariate analysis (Fig. 1). Accordingly, the simultaneous representation of scores and loadings gave a global view of the main attributes responsible to fish group dispersion. PC1, with 40% of the explained variance, is conditioned by “oily” flavour and “oily” odour, together with attributes depending on fat content such as “fatness” texture and persistence of “aftertaste”, all of them attached to the farmed group. The wild group had “seafood” flavour and “seafood” odor as the attributes that facilitate their description in reference to the PC2 (15%). Additionally “juiciness” and “shininess” were also linked to the wild group (Fig. 1).

Slight variations appeared on analysis of the attributes within each Institute panel, in spite of the univariate analysis that showed no significant effects attributable to the panel. In any case, attributes related to “oily” and “seafood” perceptions are the most important (Fig. 2). Results in the panel developed in the IMIDA show “juiciness” and “firmness” linked to the wild group. In addition, firmness, unlike the other participant panels, showed no clear correlation with the chewiness. In the three participant panels, results for “integrity” in the appearance attribute after cooking the fillet were dependent on origin, although, in the IUSA and IRTA panels this does not appear to be so significant. Precisely, these differences between panels emerged with the univariate analysis, as we can see an effect of the interaction origin/panel for the above mentioned attributes.

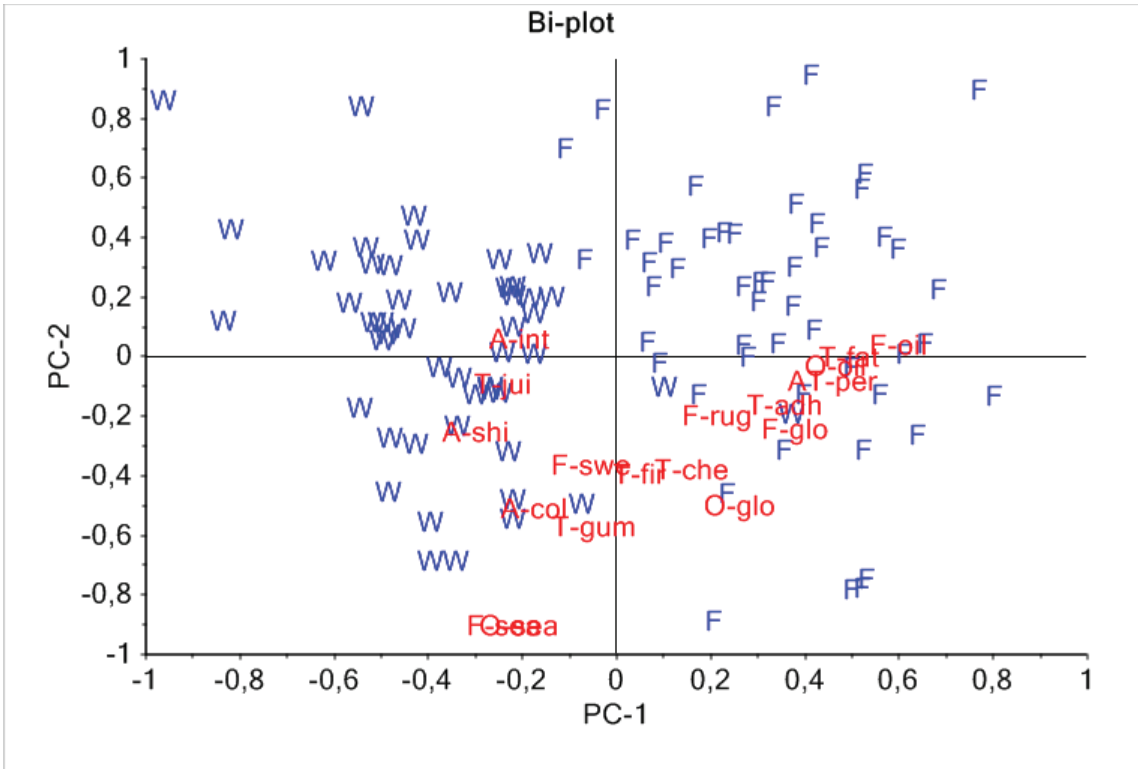


Figure 1. PCA showing distribution of sensory attributes of cooked blackspot seabream in relation with the origin: farmed (A) or wild (W).

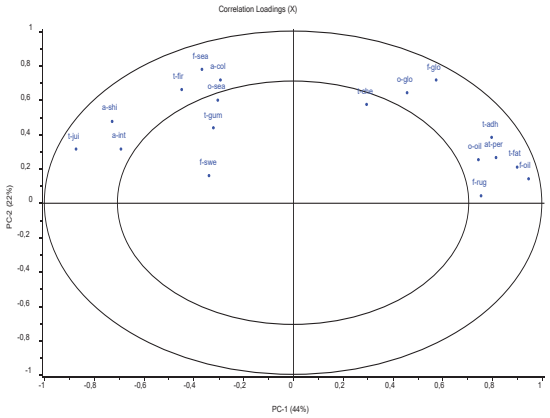
2.3.2. Proximate composition and fatty acid profile

As shown in Table 4, average intramuscular fat content of farmed blackspot seabreams was threefold higher than that of wild fish ($P < 0.000$). With regard to fat content, significantly lower moisture values were found on farmed fish ($P < 0.000$). No protein or ash differences were found associated to fish groups. On wild fish, significantly higher collagen content was determined ($P < 0.005$).

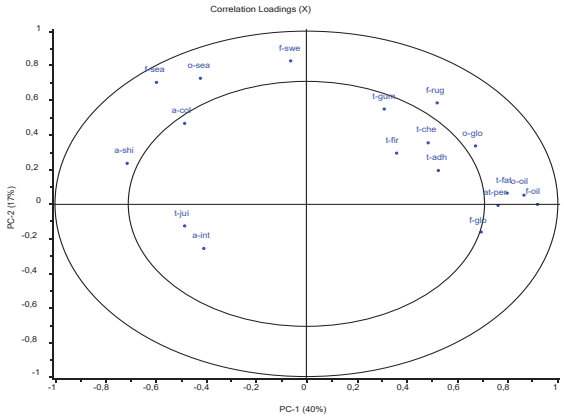
The fatty acid composition of the muscle analyzed (Table 5) shows differences associated with experimental groups. It is of note that farmed fish have a higher level of monosaturated fatty acids, especially oleic acid (C18:1*n*-9) with a higher proportion in farmed fish ($P < 0.000$). As for the omega-6 group, linoleic acid (C18:2*n*-6) in the wild fish group was tenfold lower than in farmed fish muscle and araquidonic acid (C20:4*n*-6) was significantly lower than that found in wild fish ($P < 0.000$). Linolenic acid (C18:3*n*-3) from the omega-3 polyunsaturated group was significant higher than that of the wild group. Eicosapentaenoic acid (EPA, C20:5*n*-3) and docosahexaenoic acid (DHA, C22:6*n*-3) also had an inverse relationship with the experimental group with high levels of EPA in farmed ($P < 0.000$) and high levels of DHA in wild ($P < 0.000$). These changes

on the total amount of *n*-3 and *n*-6 fatty acids reduced the n-3/*n*-6 ratio in the farmed fish group ($P < 0.000$).

a.



b.



c.

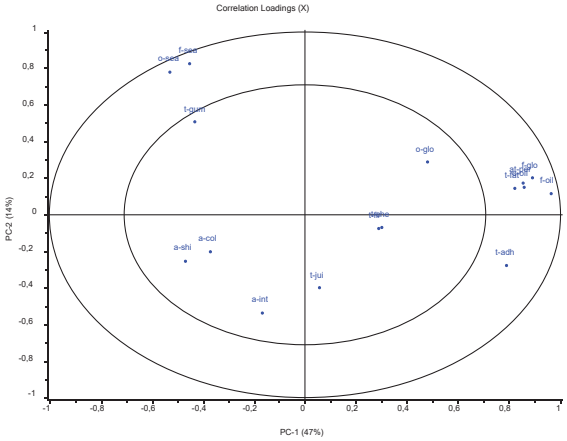


Figure 2. Correlation loadings of PC1 vs. PC2 in each panel: IMIDA (a), IRTA (b) and IUSA (c). Ellipses mark the 50% and 100% explained variance limits.

Table 4. Proximate analysis (%) in raw flesh of farmed and wild blackspot seabream. Values are expressed as means ± SD. n=30

	Wild	Farmed	P-value
Fat	0.81 ± 0.13	2.40 ± 0.26	0.000
Moisture	78.50 ± 0.25	76.19 ± 1.31	0.000
Ash	2.15 ± 0.50	2.15 ± 0.31	0.970
Protein	18.89 ± 0.59	19.02 ± 1.89	0.090
Collagen	1.40 ± 0.13	1.14 ± 0.21	0.005

Table 5. Fatty acids profile (% of total fatty acids) in raw flesh of farmed and wild blackspot seabream and probability values. Values are expressed as means ± SD. n=30

Fatty acid	Wild	Farmed	P-value
C14:0	1.26 ± 0.39	3.66 ± 0.47	0.000
C16:0	18.84 ± 0.73	20.06 ± 1.80	0.063
C17:0	0.28 ± 0.04	0.57 ± 0.04	0.000
C18:0	6.45 ± 0.23	6.48 ± 1.71	0.956
Σ saturated	27.63 ± 0.45	31.34 ± 3.94	0.008
C16:1n-7	2.18 ± 0.34	4.73 ± 0.54	0.000
C18:1n-9	7.81 ± 1.88	19.55 ± 1.36	0.000
C18:1n-7	1.45 ± 0.14	2.60 ± 0.46	0.000
C20:1n-9	1.16 ± 0.33	1.17 ± 0.15	0.000
C22:1n-11	0.24 ± 0.15	0.72 ± 0.10	0.000
Σ monoenes	13.86 ± 3.05	30.46 ± 2.37	0.000
C18:2n-6	1.28 ± 0.39	12.77 ± 0.99	0.000
C20:2n-6	0.23 ± 0.06	0.47 ± 0.03	0.000
C20:4n-6	4.11 ± 0.46	0.76 ± 0.08	0.000
Σ *n*-6 FA	10.42 ± 0.79	15.40 ± 1.22	0.000
C18:3n-3	0.22 ± 0.06	1.17 ± 0.08	0.000
C18:4n-3	0.25 ± 0.05	0.44 ± 0.07	0.000
C20:4n-3	0.38 ± 0.05	0.54 ± 0.05	0.000
C20:5n-3 EPA	3.48 ± 0.23	5.06 ± 0.87	0.000
C22:5n-3	2.46 ± 0.19	3.32 ± 0.52	0.000
C22:6n-3 DHA	39.12 ± 2.79	9.33 ± 1.98	0.000
Σ *n*-3 FA	46.41 ± 2.54	20.99 ± 3.53	0.000
Ratio Σ *n*-3/Σ *n*-6	4.46 ± 0.32	1.36 ± 0.22	0.000
HUFA n-3	45	18	

Table 6. Texture parameters in whole fish, raw and cooked flesh of farmed and wild blackspot seabream. Values are expressed as means ± SD. n=6

	Wild	Farmed	P-value
Whole fish			
Hardness	1.93 ± 0.19	1.77 ± 0.48	0.501
Springiness	0.98 ± 0.02	0.97 ± 0.01	0.488
Raw flesh			
Fracturability	4.50 ± 1.46	2.72 ± 0.20	0.027
Hardness	6.81 ± 1.74	4.79 ± 0.50	0.038
Springiness	0.25 ± 0.06	0.30 ± 0.03	0.116
Adhesiveness	-0.12 ± 0.05	-0.09 ± 0.04	0.357
Cooked flesh			
Hardness	19.10 ± 5.11	14.60 ± 4.26	0.169
Springiness	0.34 ± 0.01	0.42 ± 0.07	0.037
Adhesiveness	-0.55 ± 0.24	-0.33 ± 0.20	0.387

2.3.3. Texture and colour

Texture values in whole ungutted fish showed no significant differences in the parameters used to compare the experimental groups. However, raw fillet texture in the wild fish group showed significantly higher fracturability ($P < 0.05$) and hardness values ($P < 0.05$) compared to the farmed group (Table 6). In cooked fillets, springiness was higher in the farmed group compared to the wild ($P < 0.05$, Table 6).

Colour parameters measured by means of a colorimeter on the fish skin (Figure 3) showed significantly higher values in the wild fish group for lightness (L*, $P < 0{,}001$) and redness (a*, $P < 0{,}000$). In spite of this high value of a*, the colour saturation, measured as chroma, showed no significant differences due to a balance with b* values. Hue values of the skin were found to be lower ($P < 0{,}000$) within the wild group (57.85 ± 8.47 wild; 80.83 ± 3.22 farmed). As for muscle colour (Figure 4), significant differences were found in b* ($P < 0{,}000$) and Hue values ($P < 0{,}007$) with higher values in the farmed fish

group (284.04 ± 8.04 wild; 296.63 ± 4.14 farmed), while chroma was found to be higher in the wild group (*P* < 0,000).

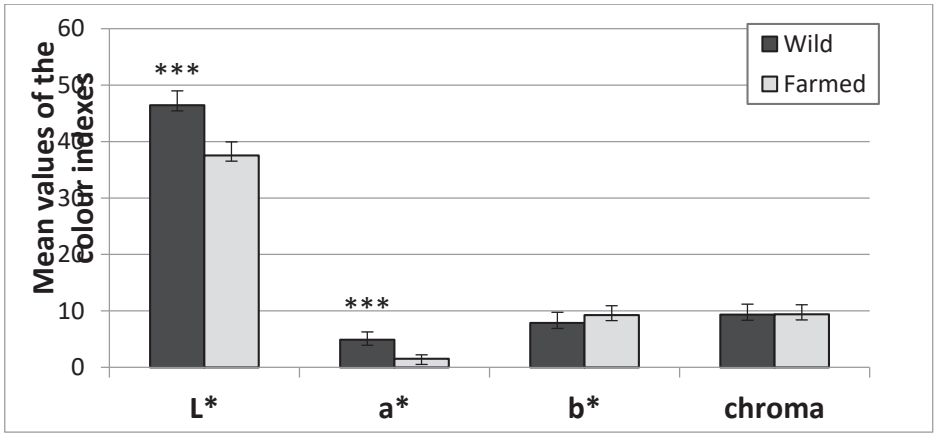


Figure 3. Analysis of colour parameters (L*. a*. b*) on skin of farmed and wild blackspot seabream. Significance level: *P* <0.001= ***; *P<0.01=**.*

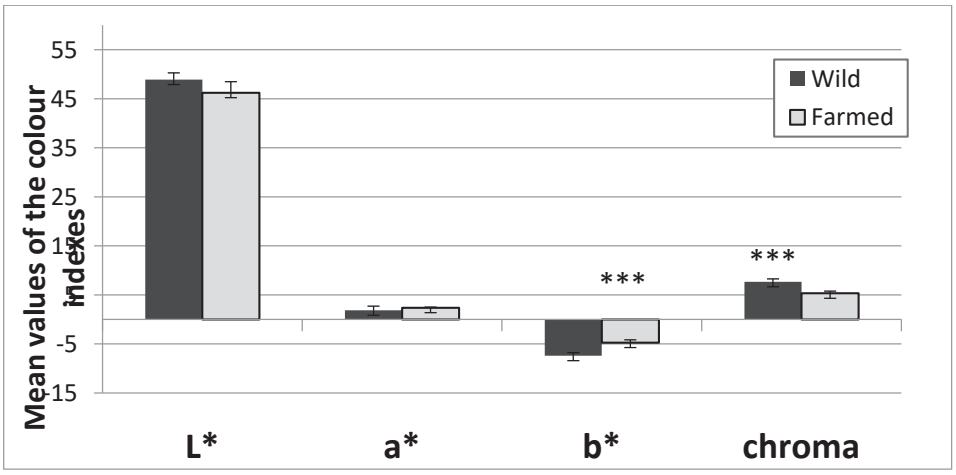


Figure 4. Analysis of colour parameters (L*. a*. b*) on raw flesh of farmed and wild blackspot seabream. Significance level: *P* < 0.001= ***; *P* < 0.01=**.

2.3.4. Muscle fibres

The extension of type I red muscle in farmed fish, beneath the subcutaneous level, was significantly higher in the cranial, medial and caudal sections (Figure 5, *P* < 0.001), and this difference was visually noticeable. Fibre count per mm^2 measured with an image analyzer showed that the number in the red muscle of the wild group (Figure 7) was higher than that of the farmed group (Figure 8) in all studied areas (*P* < 0.001). The

number of type II white muscle fibres was similar between wild and farmed fish in the medial and caudal sections but higher in the cranial section of the wild fish group (Figure 6, $P < 0.001$). Morphologically, compared with white type II fibres, red type I fibres were smaller, more uniform in size and with a more rounded shape than white fibres, which consist of small diameter fibres intermingled with larger fibres, giving the muscle its characteristic mosaic appearance.

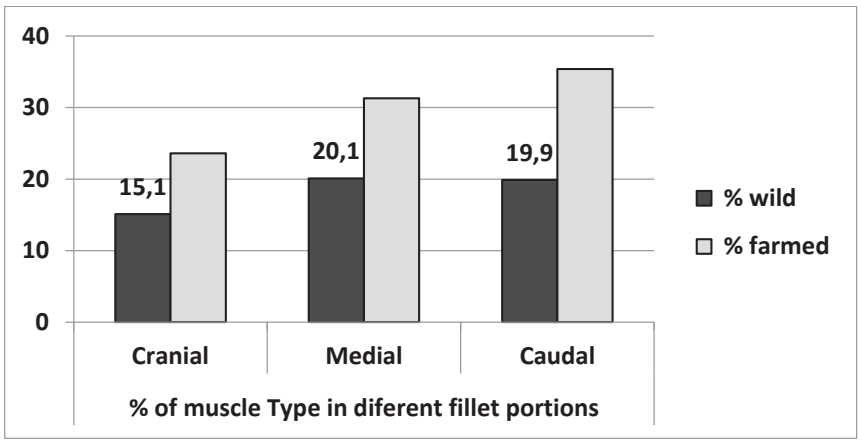


Figure 5. Analysis of the proportion (%) of muscle type (red or white) in cranial, medial and caudal portion of the blackspot seabream fillet. Significance level: $P < 0.001$= ***; $P < 0.01$=**.

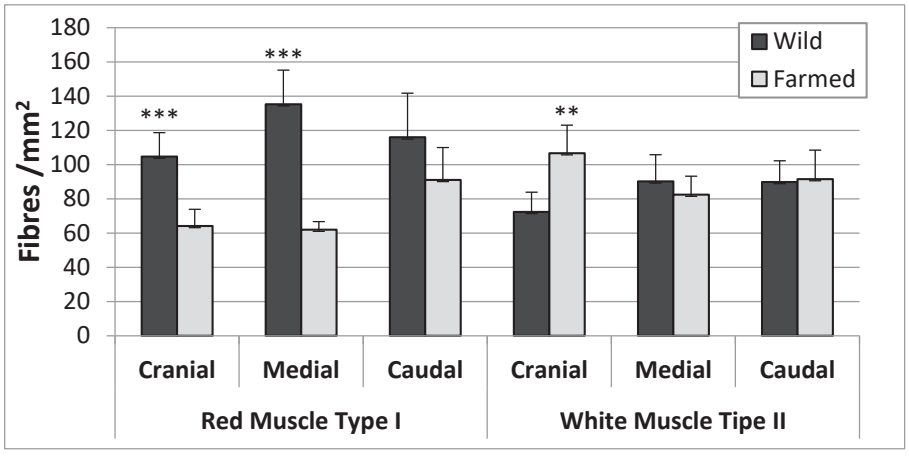


Figure 6. Analysis of fibres amount (red or white) per mm^2 in cranial, medial and caudal portion of the blackspot seabream fillet. Significance level: $P < 0.001$= ***; $P < 0.01$=**.

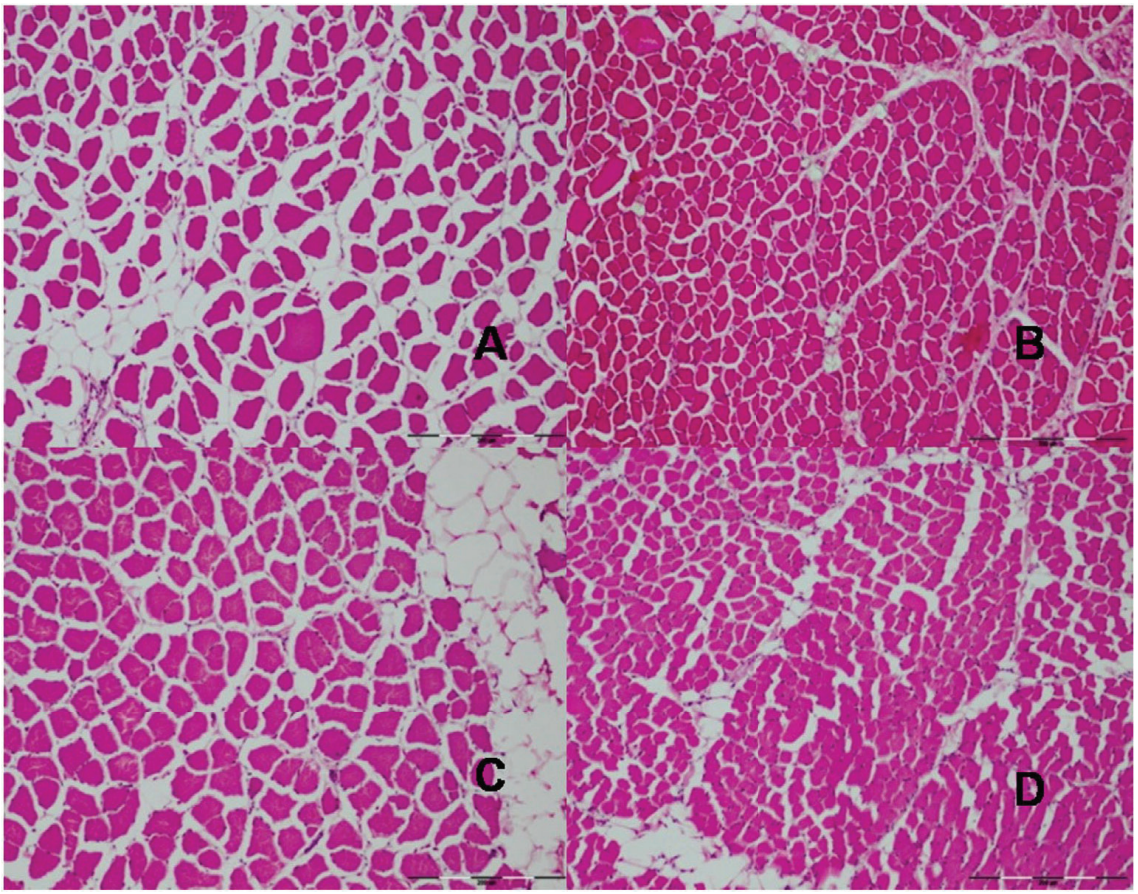


Figure 7. Red fibres amount of the blackspot seabream fillet in two portions (cranial and caudal). Haematoxylin-eosin 10x. **A.** Farmed fish, red muscle from the cranial portion of the fillet. **B.** Wild fish, red muscle from the cranial portion of the fillet. **C.** Farmed fish, red muscle from the caudal portion of the fillet. **D.** Wild fish, red muscle from the caudal portion of the fillet.

2.4. DISCUSSION

2.4.1. Sensory evaluation

Noticeable differences in sensory attributes in farmed and wild blackspot seabreams were associated with the “oily” flavour, “oily” odour, “fatness” texture and “persistence” of aftertaste. All these attributes describe fat content variations in flesh and accordingly high scores were apparent in the farmed group. With no clear differences in flesh fat level, Valente *et al*. (2011) found a significant relationship between lipid content and both fatty flavour and the perception of fatty texture after comparing gilthead seabreams from different production systems. In the present study, the correlation loading of the multivariate analysis suggests that the majority of the overall differences found between the farmed and wild groups, for all three panels, can be ascribed to differences in the

same few attributes. Fish farming increases lipid content, and so the effect on perception of oiliness will be more intense in lean species, as was previously shown in the red porgy (García-Romero *et al*., 2014), when variations in diet modified flesh lipid content. Global odour and flavour intensity are both dependent on flesh lipid content. Thus, the intensities registered are also linked to fat increase, and are greater in farmed versus wild fish. In turbot, ventral fillets showed a more pronounced odour than dorsal ones, probably due to the relatively higher fat content of the ventral fillet (Regost *et al*., 2003). Lipid content could affect assessors' perception and this could have had an influence over "seafood" scores. In this sense, both "seafood" odour and "seafood" flavour were key in the description of the sensory profile in wild fish. In farmed fish, in spite of having high scores for those attributes, global odour intensity and "oily" odour could fade "seafood" perception. Together with fat content, fatty acid composition maintains a close relationship with aroma-active compounds of muscle (Sérot *et al*., 2001). Thus, organoleptic differences can be related to fatty acid profiles (Grigorakis *et al*., 2003) since most of the volatile compounds in fish are derived from the oxidative breakdown of unsaturated fatty acids (Sérot *et al*., 2002; Grigorakis, 2007). Hexanal has been described as the main volatile compound to contribute to seafood-like flavour (Laohakunkit *et al*., 2014). However, sensory differences were detected between farmed and wild barramundi (*Lates calcarifer*) with similar hexanal concentration (Frank *et al*., 2009), which leads us to assume that complex products with complex flavour combinations of volatiles may yield different perceptions to those expected from individual compounds (Chambers & Koppel, 2013). In fact, differences in taste found in wild gilthead seabreams could possibly be related to the higher number of volatile flavour-contributing compounds observed (Alasalvar *et al*., 2005).

Fillet lipid content has a connection to flesh texture (Johansson *et al*., 2000) and it affects texture attributes, mainly fatness. This was determined by comparing farmed and wild gilthead seabreams (Orban *et al*., 1997) and salmon, after increasing the fat level in the diet (Einen and Thomassen, 1998). Additionally, fatty acid fillet composition seems to be linked to fatness and the juiciness experience (Waagbo *et al*., 1993). Izquierdo *et al.* (2003) concluded that a slightly lower hardness found in the fillet from gilthead seabreams fed vegetable oils may have been related to the slightly higher lipid content and significantly lower percentage of saturated fatty acids found in those fish. Other authors (Turchini *et al*., 2003) found no significant differences between the two parameters firmness degree during chewing and resistance to force applied in the mouth, in cooked fillet sensory texture despite the differences determined in lipid content and fatty acid profile. In the blackspot seabream, "firmness" was similar in wild and farmed

fish. However, differences in other texture attributes such as juiciness and gumminess were significantly higher in the wild group, while farmed samples achieved higher scores in chewiness and adhesiveness. Similar results from wild gilthead seabreams were described by Grigorakis *et al*., (2003), where wild fish were juicier due to higher water content, since juiciness is related to the impression of moisture running out of the meat as pressure is applied by the teeth. Conversely, Orban *et al*. (1997) found the flesh of farmed gilthead seabreams juicier, fattier and less fibrous than the wild. Fat-rich tissues usually taste smooth and succulent (juicy), whereas when fat levels are low, the sensation of driness or fibrousness (rough or coarse) describes the tissue better.

Lipid content, water content and fibre characteristics are thought to contribute to the juiciness of the fish in organoleptic tests (Johnston, 1999). This was shown in the Atlantic salmon (Bjerkeng *et al*., 1997), relating juiciness to the water-holding capacity and fat content of the fillets influenced by the fatty acid composition. In gilthead seabreams, the substitution of fish oils in their diets for vegetable oils promoted changes in flesh fatty acids. It resulted in a higher juiciness and adhesiveness when a soybean oil diet was provided, since soybean oil was found to be related to lower saturated and higher total polyunsaturated fatty acid content in muscle. It was also observed in this study that diets composed exclusively of fish oil lead to lower water content in the fillets (Izquierdo *et al*., 2005).

The highest appearance scores were achieved by wild fish fillets, showing significant differences for all attributes. Using halibut as a model, Olsson *et al*. (2003) found higher values in whiteness in fish from farmed origin. The low pH in the muscle can contribute to increase whiteness by protein aggregation and possibly expelling of intrafibrillar water. Muscle fat levels have also been reported to be involved in the white appearance (Grigorakis *et al*., 2003; García de la Serrana *et al*., 2013). Comparing different diets and flesh appearances, Izquierdo *et al*. (2005) found that gilthead seabreams fed with rapeseed oil showed a significantly more yellow colour than those fed with fish oils, suggesting that dietary lipid composition affects flesh colour. In farmed gilthead seabreams, a more white appearance was found in muscle compared to wild fish, due to the higher fat content in farmed fish (Grigorakis *et al*., 2002).

2.4.2. Proximate composition and fatty acid profile

When comparing blackspot seabream experimental groups, samples from the farmed source presented a significantly higher fat content, mainly due to a high dietary fat level

in their food and their relatively reduced activity. Additionally, when the studies are performed on lean species which have less than 1% fat in a wild environment, fat content variations are more noticeable (Sérot *et al*., 1998; Martínez *et al*., 2010). Collagen content was significantly lower in the farmed group confirming that plant protein sources in aquaculture food contain lower levels of hydroxyproline (Aksnes *et al*., 2008). Hydroxyproline, which is obtained from the diet allows collagen deposition on muscle (Zhang *et al*., 2013).

The percentage of total saturated and monoenoic acids was higher in farmed compared to wild blackspot seabreams, whereas the total polyenoic content was lower, probably due to the high content of monoenoic fatty acids in the diet of the cultured fish. It has been reported that assimilation patterns of dietary fatty acids in fish muscle reflect the content of the dietary lipid sources (Grigorakis *et al*., 2002; Pirini *et al*., 2000). Levels of saturated fatty acids (SFA) are low in farmed species compared to their wild counterparts, including gilthead sea breams (Grigorakis *et al*., 2002) and sea bass (Alasalvar *et al*., 2002b). However, in farmed blackspot seabreams unusual proportions of saturated fatty acids were found, similar to species such as the salmon or *rainbow trout* (*Oncorhynchus mykiss*) (Blanchet *et al*. 2005). Palmitic acid was the principal SFA, contributing approximately 64 to 68% of the total SFA content of the lipids for both farmed and wild blackspot seabreams. Similar results were found for gilthead seabreams (Grigorakis *et al*., 2002), sea bass (Alasalvar *et al*., 2002a) and red porgy (Rueda *et al*., 1997). Oleic acid was identified as the primary monoenoic fatty acid. In farmed sea bass and seabreams this has been reported to arise from its dominance in the commercial feed (Grigorakis *et al*., 2002). Among the n-3 series, DHA values in the wild group were one of the highest among related farmed species (Rueda *et al*., 1997; Grigorakis *et al*., 2002; Alasalvar *et al*., 2002b). In the blackspot seabream, as a lean species, DHA is high in relation to total fat content. In the farmed fish group the values were close to that found in salmon (Bell *et al*., 2003) and similar to those from related species (Grigorakis *et al*., 2002; Alasalvar *et al*., 2002b; Rueda *et al*., 1997). EPA values in farmed fish were paradoxically higher than in wild fish, in contrast with those found in other farmed species. This fact implies that a fillet portion of 100 g has a HUFA (EPA+DHA) content of 347 mg/100 g in wild fish quite similar to the 359 mg/100 g in farmed blackspot seabreams. Farmed fish have a higher level of linoleic acid than wild blackspot seabreams. This fatty acid is present in plant oils used in the feeding of cultured fish, especially with soya, the only vegetable oil present in the experimental diet. It is accumulated largely unchanged in the lipids of marine fish due to their reduced capacity for chain elongation and desaturation. The level of araquidonic acid in farmed fish is low

since the fish oils used to formulate the diet contain minimal amounts of this fatty acid. In wild fish the level was also low compared to related species such as the gilthead seabream (Grigorakis *et al*., 2002), sea bass (Alasalvar *et al*., 2002a), or red porgy (Rueda *et al*., 1997).

2.4.3. Texture and colour

Collagen level has an effect on muscle firmness. Thus, the higher values of collagen content in the raw fillet of wild fish result in a higher force needed to compress and fracture it, which is reflected in the sensory profile. Texture studies on whole raw fish are valuable as they involve a non destructive procedure related to habitual commercial determinations in markets or retailers (Ginés *et al*., 2002). At this stage no differences were found, probably due to freshness and the scaffolding effect of skin, which gives structure and shape to the whole raw fish. Raw fillet hardness varies with biological factors including size, diet, storage time and temperature, whereas fat content does not appear to affect instrumental hardness values (Castro *et al*., in press). Muscle consists of two major components: connective tissues of the myocommata and the extra-cellular matrix, and the intracellular contractile proteins (mainly actimyosin). To determine hardness, 80% of the fillet has to be compressed producing a loss of intrafibrillar integrity, especially affecting actimyosin, which may influence the hardness values in extensively cultured fish (Ingolfsdóttir, 1997). Together with the amount of collagen, it was concluded in salmon that firmness of muscle was not related to the total amount of collagen in the muscle, but rather to higher collagen stability (Moreno *et al*., 2012). Firmness was evaluated but no differences were found (results not presented). Fracturability, on the other hand, depends mainly on the collagen content. In this sense, wild fish fillet requires more force to change the shape of the sample than farmed fish fillet, due to higher collagen content. In other species such as the gilthead seabream, Alasalvar *et al.* (2002b) did not report hardness differences between raw fillet of wild and farmed fish. White muscle cellularity is an important determinant of the textural characteristics of the flesh and several studies have found a relationship between muscle fibre size and the firmness of the flesh (Hatae *et al*., 1990; Hurling *et al*., 1996). Texture was determined in fillet pieces from the first third of the fillet, where fibres were found with different diameters between groups. Hurling *et al.* (1996) stated decreasing sensory firmness with increasing fibre cross-section which could also influence the taste and processing characteristics of the flesh (Johnston, 1999). Texture parameters such as hardness and springiness in cooked fillets did not depend on the connective tissue proteins. After

cooking the collagen responsible for maintaining the structure of the fillet, it was gelatinized by thermal action (Castro *et al*., in press), whereas the actomyosin complex changes from a soft gel to a firmer denatured complex (Dunajski 1979).

Ocano-Higuera *et al*. (2009) reported that colour is one of the most important parameters used to evaluate the quality of fishing products. Wild blackspot seabreams have a very particular vivid red-pink-silver colour in the dorsal area and fins, which is very valuable as a descriptor of the skin colour. An orange global appearance is valued by consumers. In red porgy (*Pagrus pagrus*) studies have found that the red to yellow pigment ratio has an importance in consumers' appreciation (Kalinowski *et al*. 2011). Skin results showed higher b* values both in the wild and farmed group. However, a*/b* ratios were higher for wild fish giving a distinctive orange skin colour. In farmed fish the a*/b* ratio moved towards the yellow zone of the colour spectrum, confirming that farmed fish skin has a yellowness character that can compromise consumer acceptance. In the gilthead seabream it has been found that different rearing conditions affect lightness and skin colour distribution (Gines *et al*., 2004; Valente *et al*., 2011). Farmed fish, reared in sea cages, have darker skin than their wild counterparts as they are more exposed to solar light and so their skin chromophores become darker (Adachi *et al*., 2005). Differences are related not only to rearing conditions but also diet composition. Montero *et al*. (2005) found differences in source and level of lipids in muscle, and specifically higher yellowness in flesh coloration when compared with fish fed with the 100% fish oil diet. In salmon, soya oil inclusion into the diet promoted differences in lightness (L*), redness (a*) and Hue of the muscle (Rørå *et al*., 2005).

2.4.4. Muscle fibres

Cellularity is frequently used as an indicator of changes in the hypertrophy–hyperplasia balance, which is also an important determinant of fish flesh quality and is thus of applied economic relevance (Johnston *et al*., 2000). Type II white muscle from the fillet of the seabream blackspot, which comprises the major edible part of the myotome, was found to have different diameter fibres on the first third of the farmed fish fillet. Fibre density was significantly lower in the farmed than in the wild group reflecting a different pattern of muscle growth, with a low rate of white muscle fibre hyperplasia and body growth. Oxidative type I muscle fibres are devoted to relatively continued movement during slow swimming or cruising whereas glicolitic type II white muscle is related to bursts of fast movements associated with a free lifestyle. Wild blackspot seabream fisheries have a

seasonal character which suggested a species migratory movement (Pinho *et al*., 2014) differing from the farmed sedentary setting. The recruitment of type I fibres of the red muscle in wild fish was more important that in farmed fish, through a hyperplasia process. This fact is connected to fish lifestyle, where farmed fish are confined in a smaller space, (Totland *et al*., 1987) different water temperature (Johnston *et al.*, 2003; Ayala *et al*., 2000, 2001; López-Albors *et al*., 2003), dietary protein intake and age. The latter, age, is an important factor because hypertrophy–hyperplasia phenomena are dynamic during seabream blackspot growth (Silva *et al*., 2008). This morphological difference could also be related to the two different subtypes of slow red muscle based on myosin inmunoreactivity that has been described in the blackspot seabream (Silva *et al*., 2008). In other species such as sea bass, similar results were described in white muscle fiber diameter (Periago *et al*., 2005). The presence of a mayor amount of collagen in the wild group is also related to fibre proportions (Montero & Borderías, 1990). Collagen is the most abundant protein in intramuscular connective tissue, since it is a major component of the endomysium. The collagen level determined in the wild group was higher in the textural profile particularly in hardness and related parameters. These differences were less accentuated in cooked fish because thermal processes solubilize collagen, as stated above.

The results of the present study show compositional, sensory and instrumental variations mainly in colour, texture and fibre composition of the blackspot seabream. An unusual fatty acid composition especially with regards to HUFA content was maintained at healthy levels under farming conditions.

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CAPÍTULO 3. Blackspot seabream (*Pagellus bogaraveo*) fed different diets. Histologic study of the lipid muscle fiber distribution and effect on quality during shelf life

Aquaculture, 484, 71-81

Abstract

To determine differences in commercial size between wild and farmed blackspot seabream, some parameters affecting quality were evaluated during shelf life, namely the fatty acid profiles, pH, muscle colour and Quality Index Method (QIM). In addition, a histological study of the distribution of the lipids in the myotome (slow or fast twitch) in different dorsal areas was performed. In farmed fish, three diets were tested. One of them was a commercial diet formulated with a high proportion of lipids, mostly from fish oil. The other two diets were formulated to reduce lipid levels by 60%. In one (low lipid diet, LL) the proportions of protein sources were maintained and in the other (low lipid diet + fish meal, LL +) most of the protein was provided as fish meal.

Lipid depots were found not to be different in the myocytes of the fast-twitch fibers. However, regional differences were identified in the slow-twitch fibers depending on the area and the diet, whether in the cranial, medial or caudal portion of the dorsal myotome. The worst scores of the QIM were recorded for the Control diet and the wild fish group. In addition, QIM showed a positive correlation with total *n*-3 and a negative with other lipid fractions. The a* values in skin and the chroma in muscle of the wild fish were superior to those measured in farmed fish. Fish fed the LL+ diet had higher protein content in the fillet than that in the wild counterpart. As for nutritional value, farmed fish were able to deposit more DHA than was offered in all experimental diets used, especially with the LL+ diet which yielded threefold more DHA.

3.1. Introduction

The diversification of the seafood market in southern Europe demands that traditional production of species such as the gilthead seabream (*Sparus aurata*) and the European seabass (*Dicentrarchus labrax*) give up part of their international prominence to new finfish species. Because of its high commercial value due to the firmness and the flavour of its flesh, scarcity in the market and biological characteristics, the blackspot seabream (*Pagellus bogaraveo*) has become a suitable alternative for the markets (Peleteiro *et al*., 2000). This is especially the case considering that this semi-pelagic marine fish, commonly found in the European coasts, is being overfished and due to the collapse of traditional fisheries (Lorance, 2011).

Studies of the nutritional dynamics of the blackspot seabream have focused on the effects of different feeding levels on growth response, body composition, and energy expenditure of blackspot seabream juveniles (Peleteiro *et al*., 2000; Olmedo *et al.,* 2000;

Linares *et al*., 2001, Silva *et al*., 2006; De Almeida Ozório *et al*., 2009). Thus, it is suggested that the blackspot seabream is less efficient than other sparidaes at converting nutrients, since it has higher dietary protein energy demands and favours lipid deposition (Silva *et al*., 2006). Commercial diets for this species have a minimum of between 45 and 50% protein content to ensure the best growth ratio, but less than half of this protein is provided by fish meal. The growth potential could therefore be diminished with the inclusion of a source of protein different from the fish meal (Figueiredo-Silva *et al*., 2010). In addition, it is common to reduce the cost of the diets through the reduction of fish oil inclusion, but this must be accompanied by an increase in energy availability via the inclusion of carbohydrates, only effective in maintaining growth parameters when the protein source comes from fish meal (Valente *et al*., 2010). Therefore, the economic profit achieved by reducing the levels of fish oil must be balanced by the required reduction in vegetable protein to complete optimal levels of growth and proper use of the different types of carbohydrates included in the diet (Figueiredo-Silva *et al*., 2009; Valente *et al*., 2010).

As stated above, the studies on blackspot seabream have been done mainly on juveniles (Silva *et al*., 2006; Parmigiano *et al*., 2007). However, these dietary changes should be made to result in fish with as similar a presentation as consumers actually would find in the market when they make the purchase decision, that is, in commercial sized fish and ice-stored fish. In these commercial conditions and regarding muscle conformation, sensory assessment, skin or muscle colour are essential as it could have a significant influence on product acceptability. Particularly, the skin pigmentation pattern can be considered an index of animal welfare in aquaculture species and a factor of economic consideration, as a pale skin colour indicates raw material quality or long storage, especially important in the case of species with a distinctive red-pink skin colour that acquire darker coloration under intensive rearing conditions (Pavlidis *et al*., 2006).

Accordingly, the present work focuses on the investigation of variations in the lipid content of farmed blackspot seabream muscular fibers, freshness and nutritive composition during chilled storage, as a result of different diets and comparing them to their wild counterparts.

3.2. Materials and methods

3.2.1. Growth trial

Experiments were conducted following FELASA category C recommendations and according to the European Economic Community animal experimentation guidelines Directive of 24 November 1986 (86/609/EEC). Blackspot seabream (*Pagellus bogaraveo*) with a mean weight of 155.1 ± 30.4 g and 14 months of age from the stock maintained at the Oceanographic Center belonging to the Oceanographic Spanish Institute (Vigo, Spain) were distributed randomly in cages with 2×2 m of surface area and 8 m depth, located in Polygon B Bay (Pontevedra, Spain). 600 fish per cage were maintained at a water temperature between 13 °C in December–January and 20 °C in June–July.

3.2.2. Diets

Three diets were tested: Control, LL and LL+. The Control diet was a commercial diet (Bes-Power, Sparos, Faro, Portugal) formulated with a high proportion of lipids, mostly from fish oil, while the protein was composed two thirds from vegetable sources and one third from fishmeal (Table 1). LL and LL+ were formulated to reduce lipid levels by 60% and to compensate for the energy difference by including carbohydrates. In LL (low lipid diet) the proportions of protein were maintained and fish oil reduced. In LL+ (low lipid diet + fish meal) the reduction of fish oil was the same as in the LL diet but to improve efficiency in the use of carbohydrates, most of the protein was provided as fishmeal. The average daily feed intake was around 1.2% of body weight.

3.2.3. Sample preparation

After 20 months of feeding, a total of 30 individuals per diet, with an average weight of 551.2±46.4 g, were slaughtered by immersion in ice cold water (fish: ice ratio of 2:1). Additionally, 30 wild fish caught near the Galician Atlantic coast with an average weight of 509.0±46.8 g were purchased upon arrival at Vigo Fish Market (Pontevedra, Spain) 10 hours after being caught. The fishermen were instructed to introduce and maintain the fish freshly caught into flaked fish until port arrival. Fish were packed ungutted with flaked ice into polystyrene boxes and shipped to the laboratory, arriving within 24h after harvest. The boxes were stored at 4 ºC, replacing the ice as needed. During storage,

five randomly chosen fish per group were obtained at 1, 4, 7, 10, 14 and 17 days post-harvest (dph) and individually subjected to analytical determinations.

Table 1. Ingredients (g/kg) and chemical composition (% dry basis) of the experimental diets.

Ingredient	*Diet*		
	Control	LL	LL+
Fishmeal	270	216	456
Soybean meal	200	160	112
Wheat meal	-	187	197
Corn gluten	180	80	38
Wheat gluten	90	50	-
Bean meal	40	-	-
Pea meal	10	209	147
Fish oil	170	50	40
Soybean oil	30	6	-
Premix	10	10	10
Dicalcium phosphate	-	21	-
L-Lysine	-	8	-
DL-Methionine	-	3	-
Composition			
Crude protein	50.09	47.19	48.95
Crude lipids	29.33	12.25	12.03

3.2.4. Lipid distribution in slow and *fast-twitch skeletal muscle fibers*

Five fish (left side) per experimental group were studied on the 1st dph. Samples from the cranial, medial and caudal sections of the muscle tissue under the lateral line were obtained. Two different blocks 2 mm thick per studied area were prepared, one including fast-twitch muscle fibers and the other slow-twitch muscle fibers. These blocks were covered with tissue freezing medium (Tissue-Tek O.C.T., Sakura Finetek, Hatfield, PA), snap frozen over liquid nitrogen, wrapped in foil and stored at −80 °C until sectioning. Muscle samples were sectioned (6μm) transversely to the body axis with a cryostat (Slee, Mainz, Germany), and stained with oil red O (Lillie and Ashburn, 1943).

The proportion of lipid droplets in the different studied sections was counted with an image analysis package (Image-pro plus software, media cybernetics, Atlanta, GA) connected to a light photomicroscope (Olympus CX41, Tokyo, Japan). Three different microphotographs were randomly chosen per section at a 40× objective magnification.

3.2.5. Sensory assessment

During the experimental days, six laboratory-trained panelists evaluated sensory attributes indicative of fish freshness, applying the Quality Index Method (QIM) performed for this species (Sant'Ana *et al.*, 2011). This seafood freshness grading system applied a scale of 30 demerit points, assessing 14 parameters based on significant sensory parameters and attributes that change most significantly for raw fish and score from 0 to 3 demerit points, during degradation processes Therefore higher scores are given as the dph progresses.

The five fish per dietary group, every experimental day, were previously coded with 3-digit numbers that did not indicate the origin or diet.

3.2.6. pH and colour

The pH of the fillet was determined using a Crison penetration electrode (accurate to 0.01 pH unit, model 507; Crison Instruments S.A., Barcelona, Spain) after carrying out an incision on the skin, in the tail area. Colour of skin and fillet was recorded using a Colorimeter CR-400 (Konica Minolta, Osaka, Japan) following the system of CIE (1976), L* describing lightness (L*=0 for black, L*=100 for white), a* describing intensity of red (a*>0), b* describing intensity in yellow (b*>0), chroma (C_{ab}: $(a^{*2}+ b^{*2})^{1/2}$) and hue (H^*_{ab}: arctan (b*, a*). Skin colour was measured above the lateral line, 1 cm behind the operculum. Fillet colour was measured after removing the skin in a white muscle area. Determinations were made in triplicates, rotating the illumination system 90º after each reading.

3.2.7. Proximal composition and fatty acid profile

Unskinned fish fillets (right side) were homogenated in batches of three and immediately subjected to proximal analysis by a FoodScan™ (FOSS, Hillerød, Denmark). Ash was determined gravimetrically after combustion for 24h at 450ºC (AOAC, 1995). Lipids from diets and fillets were extracted with a chloroform: methanol (2:1 v:v) mixture as described by Folch *et al*. (1957). Polar and neutral lipids were separated by adsorption chromatography in sep-pack columns (Waters S.A., Milford, MA, USA) (Juaneda & Rocquelin 1985). Fatty acid methyl esters were obtained by transmethylation as described by Christie (1982), separated and quantified by gas chromatography under

the conditions previously described (Izquierdo *et al*., 1990) and identified by comparison to external standards. Polar and neutral lipids were determined on the 1st, 7th and 14th dph.

3.2.8. Statistical analysis

Data were analyzed with IBM SPSS Statistics for Windows, Version 21.0 (Armonk, NY: IBM Corp.). A linear model with diet and dph as fixed factors and individual weight as covariate was used to determine effects. Significant differences between the means were evaluated by Duncan's multiple range tests. Pearson correlation analysis was used to determine relationships between physico-chemical parameters and biochemical composition.

3.3. Results

3.3.1. Muscular lipid distribution

The lipid droplets in the cytoplasm of the myocytes stained with oil red O technique showed no positivity by light microscopy in the shape of red depots of the fast-twitch fibers neither when comparing dietary treatments nor on studying the different muscular areas (cranial, medial and caudal). However, in slow-twitch fiber samples, the intracellular red depots were profuse. On comparing the different studied areas of the muscle, a significantly higher amount of deposition (Figure 1) was noticed in the cranial area compared to the medial and caudal (Figure 2). As to the effect of the different experimental diets, the LL+ diet produced a greater lipid deposition but only we were able to quantify differences in the pattern of the intracellular storage from the medial and caudal areas (Figure 1 and 2).

3.3.2. Sensory evaluation

The QIM scoring on the 1st dph, on comparing experimental groups, yielded the highest values in the Control diet and in wild fish (Figure 3A). During shelf life the values increased progressively and the differences found between the Control diet and wild fish were maintained. Studying correlations between the QIM and proximal/fatty acid content in the muscle (Table 2), the Pearson coefficient showed a significant negative correlation of the QIM result with fat content and a positive correlation with moisture. Other than amount of fat, specific lipid fractions, saturated (SFA), monounsaturated (MUFA) and *n*-

6, showed a negative correlation with QIM, especially in the last dph, 14 and 17 dph. A positive correlation was found with total *n*-3.

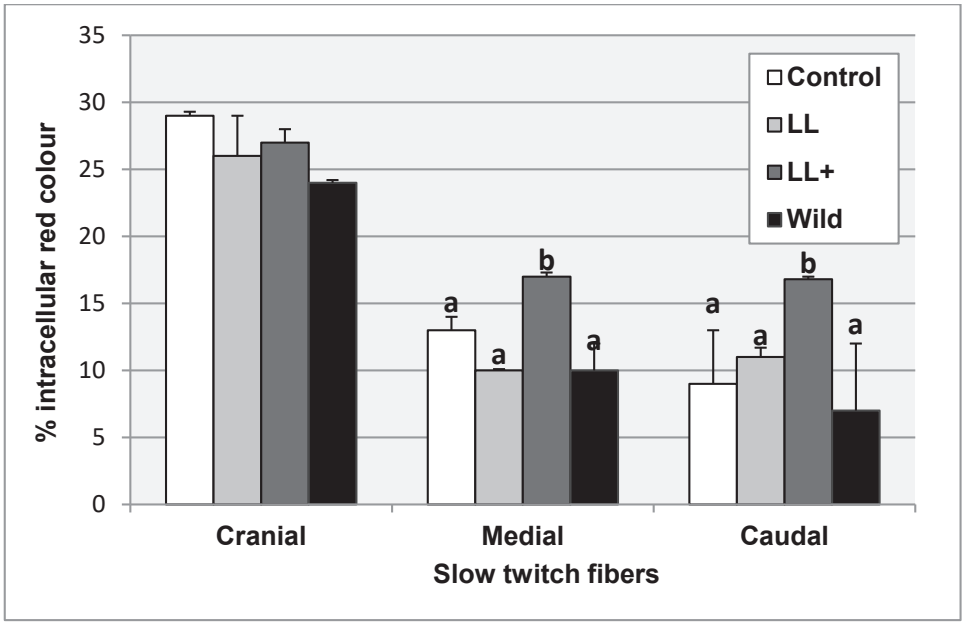

Figure 1. The percentage of intracellular lipids in the slow twitch fibers assessed with Oil Red O. Different letters in the same muscle portion (cranial, medial, and caudal) denote statistically significant differences ($P < 0.05$).

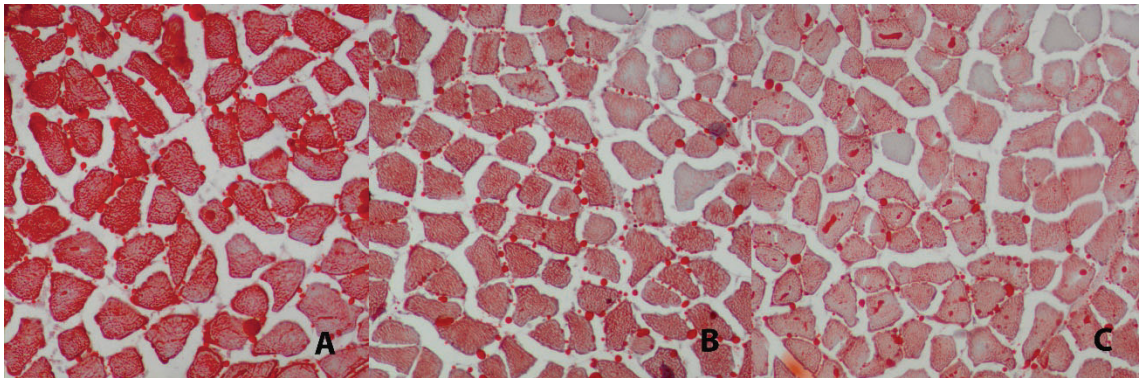

Figure 2. Representative cross-sections of the slow-twitch fibres showing different presence of lipid intracellular depots. A: cranial, B: medial and C: caudal area.

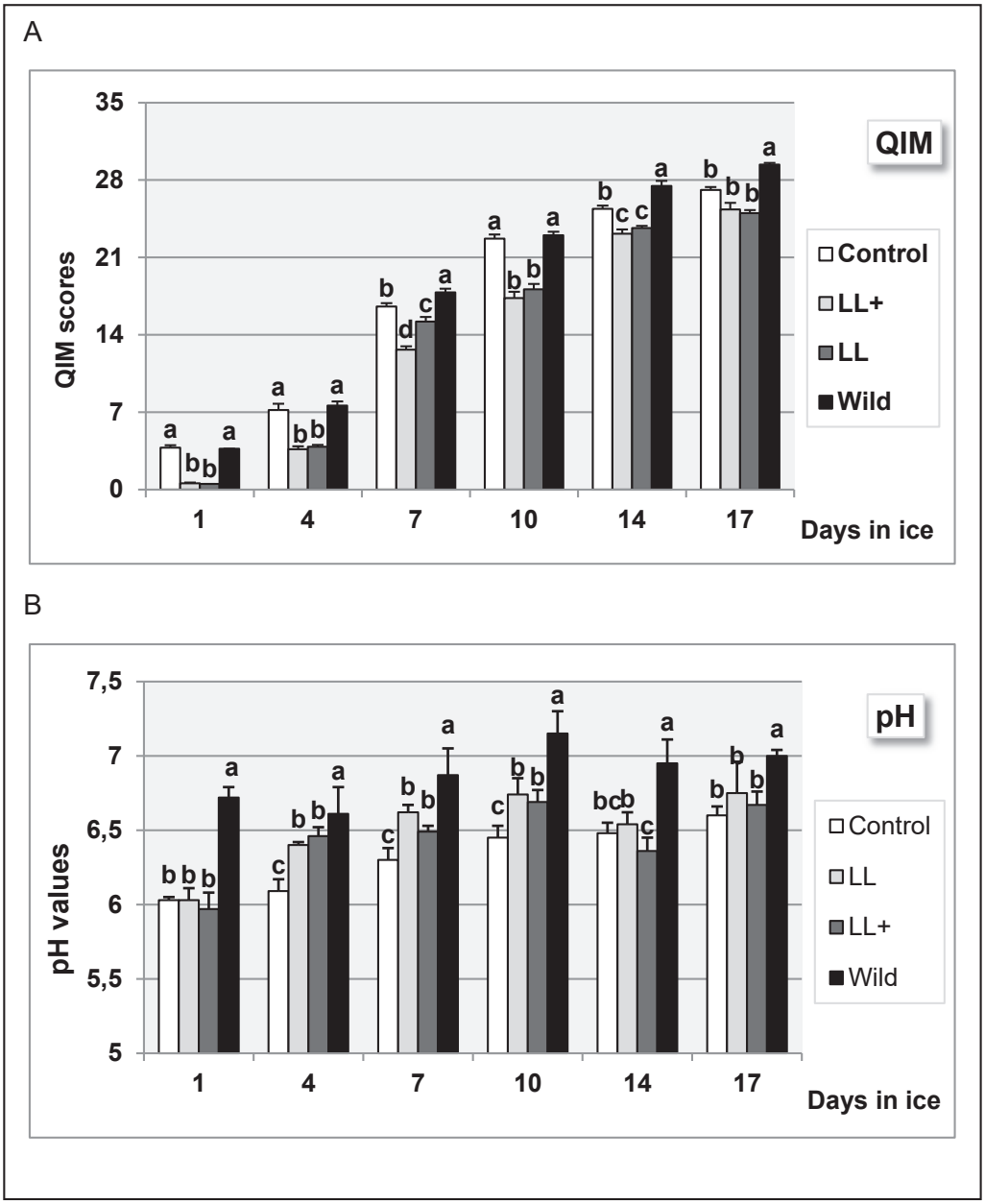

Figure 3. QIM scores in whole fish (A) and pH values in the muscle (B) of blackspot seabream, wild and fed different diets, throughout ice storage. Different letters in the same day denote statistically significant differences ($P < 0.05$).

Table 2 Pearson's correlation coefficients between QIM and chemical composition of muscle throughout ice storage (Pearson's coefficient and *P* value).

Days in ice	Protein	Lipid	Moisture	SFA	MUFA	Σ *n*-6	Σ *n*-3
1	-0.277	-0.703	0.538	-0.054	-0.541	0.307	0.334
	0.237	*0.001*	*0.014*	*0.820*	*0.014*	*0.188*	*0.150*
4	-0.026	-0.597	0.577	0.024	-0.524	-0.416	0.613
	0.912	*0.005*	*0.008*	*0.919*	*0.018*	*0.068*	*0.004*
7	-0.716	-0.437	0.525	-0.588	-0.487	-0.379	0.778
	0.000	*0.054*	*0.017*	*0.006*	*0.029*	*0.100*	*0.000*
10	-0.422	-0.460	0.556	0.032	-0.560	-0.319	0.533
	0.064	*0.041*	*0.011*	*0.894*	*0.010*	*0.171*	*0.015*
14	-0.605	-0.754	0.816	-0.305	-0.695	-0.601	0.761
	0.005	*0.000*	*0.000*	*0.190*	*0.001*	*0.005*	*0.000*
17	-0.107	-0.703	0.753	-0.568	-0.648	-0.698	0.816
	0.654	*0.001*	*0.000*	*0.009*	*0.002*	*0.001*	*0.000*

3.3.3. pH and colour

On the 1st dph, the pH values measured in the muscle of the farmed blackspot seabream were around 6.0 and not different between experimental diets (Figure 3B). The wild fish pH values, however, were significantly higher both on the 1st day in ice and during shelf life. The pH values increased throughout the dph (Figure 3B). The correlation study showed a significant relationship between pH values and muscle colour, protein, fat and moisture levels as well as with specific lipid fractions SFA, MUFA and *n*-3 (results not shown). Thus, the muscle was lighter on 1st dph with high pH values (0.667, $P = 0.001$) but from the 4th day on, the correlation was found as negative. The *b** values changed from yellow in the muscle where the pH was high (-0.830, $P < 0.000$) from the first day and during the entire period. The correlation with protein content was negative during shelf life (1st dph -0.695, $P = 0.001$; 14th dph, -0.770, $P < 0.000$). Also, it was found to be negative with lipid content (1st dph -0.674, $P = 0.001$) and MUFA (1st dph -0.946, $P < 0.000$) but positive with total *n*-3 (1st dph 0.923, $P < 0.000$).

On the 1st dph and throughout the shelf life (Figure 4A) *a** values of the skin were significantly higher for the wild group. *b** skin values on the 1st day were higher ($P < 0.05$) for the Control diet and LL+ (9.3 Control, 8.1 LL, 8.9 LL+, 7,9 Wild). During shelf life no significant trend was recorded, and only *b** values of wild fish increased ($P < 0.05$) from the 10th day in ice (results not shown). L* values studied in wild blackspot seabream skin (46.5) were found to be significant higher ($P < 0.05$) and therefore brighter than those in

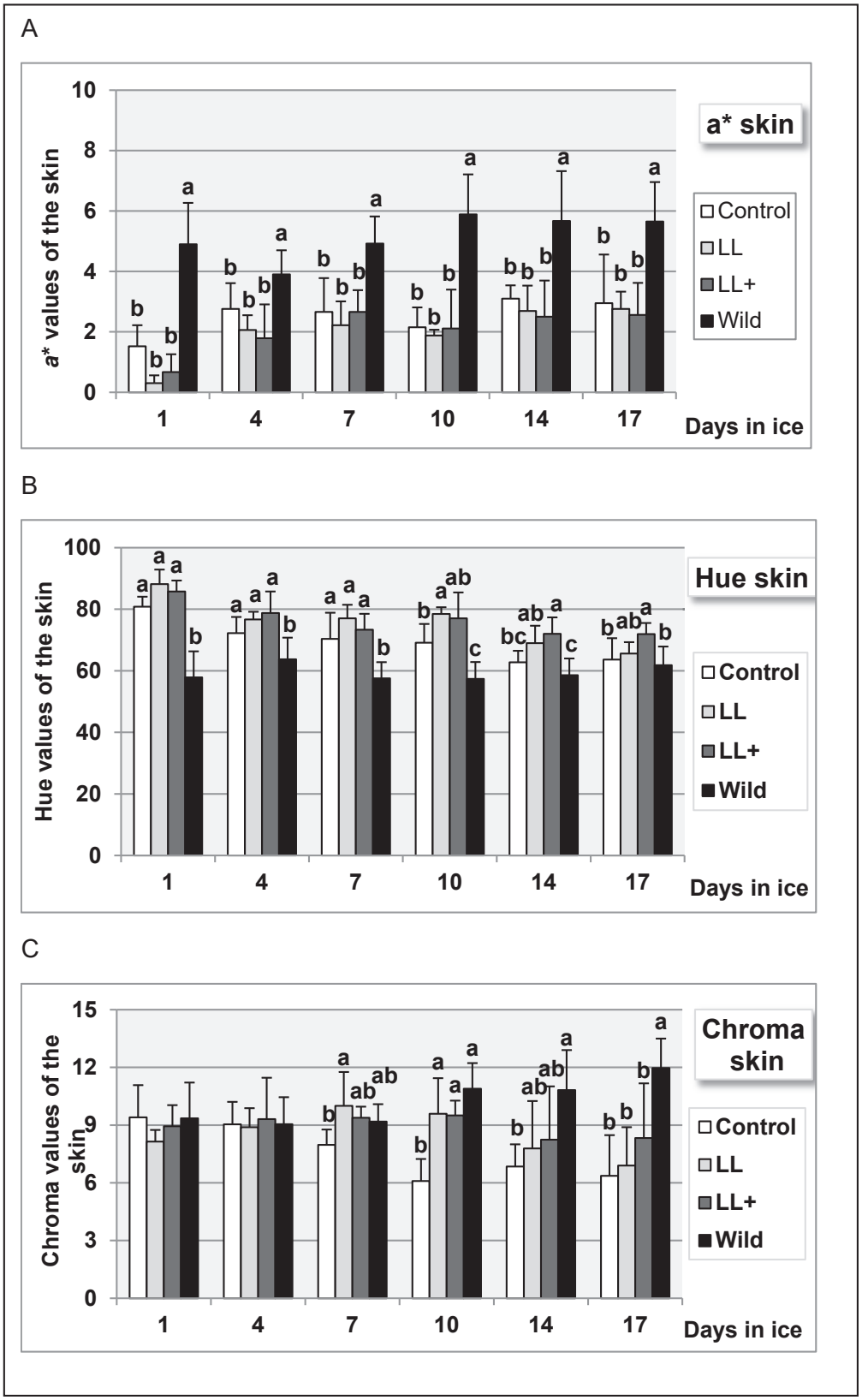

Figure 4. *a** values (A), Hue (B) and Chrome (C) of the skin of blackspot seabream, wild and fed different diets, throughout ice storage. Different letters in the same day denote statistically significant differences ($P < 0.05$).

farmed fish (37.5 Control, 40.1 LL and 38.2 LL+). This difference was not maintained throughout shelf life (results not shown). The chroma values in the skin (Figure 4B) were not different between experimental groups from the 1st dph to the 7th dph. Towards the end of the dph, the chroma values of the wild fish increased and the values of the groups fed experimental diets diminished. Compared to the fish fed experimental diets, the Hue values of the skin of the wild fish were significantly lower on the 1st dph and also during shelf life (Figure 4C). Additionally, the Hue values of the wild fish registered no significant changes during shelf life, while in the groups fed experimental diets Hue values diminished (Figure 4C).

The *a** values in the muscle on the 1st dph were found to be lower ($P < 0.05$) when feeding the LL diet (1.1) than when feeding the other experimental diets (2.4 Control, 2.3 LL+) or in wild fish (1.9). During shelf life no significant trend was recorded. The *b** values of the muscle of wild fish (-7.4) were significantly lower ($P < 0.05$) compared to the farmed groups on the 1st dph (-4.8 Control, -3.0 LL, -2.8 LL+) and throughout the dph. In all groups, *b** values decreased ($P < 0.05$) during shelf life. The L* values measured in the muscle on the 1st dph were higher ($P < 0.05$) in the wild group (48.9) than in the group fed the Control diet (46.2), and in those fed the Control diet were higher ($P < 0.05$) than those fed the other experimental diets (LL 41.8, LL+ 42.9). During shelf life a significant lightening ($P < 0.05$) of the muscle was registered. The muscular values of the chroma measured in the wild fish were found to be the highest. Throughout shelf life the chroma values of wild fish decreased (Figure 5A). Hue values in the muscle were found not to be different on the 1st dph between dietary groups. During shelf life the Hue values followed a slight increase except in wild fish that showed not significant variations (Figure 5B).

The analysis of the correlations between muscle colour and chemical composition (results not shown), showed that L* correlated on the 1st dph with protein (-0.785, $P < 0.000$) and lipid (0.739, $P < 0.000$) levels. L* was negatively correlated with saturated fatty acid levels during the shelf life period, mainly on the 1st dph (-0.700, $P = 0.001$). *b** showed a positive correlation with protein and lipid content throughout shelf life, especially for lipids on the 1st dph (0.804, $P < 0.000$). Similarly, on the 1st dph a positive correlation was found with MUFA (0.818, $P < 0.000$) and a negative one with *n*−3 Long Chain-Polyunsaturated fatty acids (LC-PUFA -0.714, $P < 0.000$).

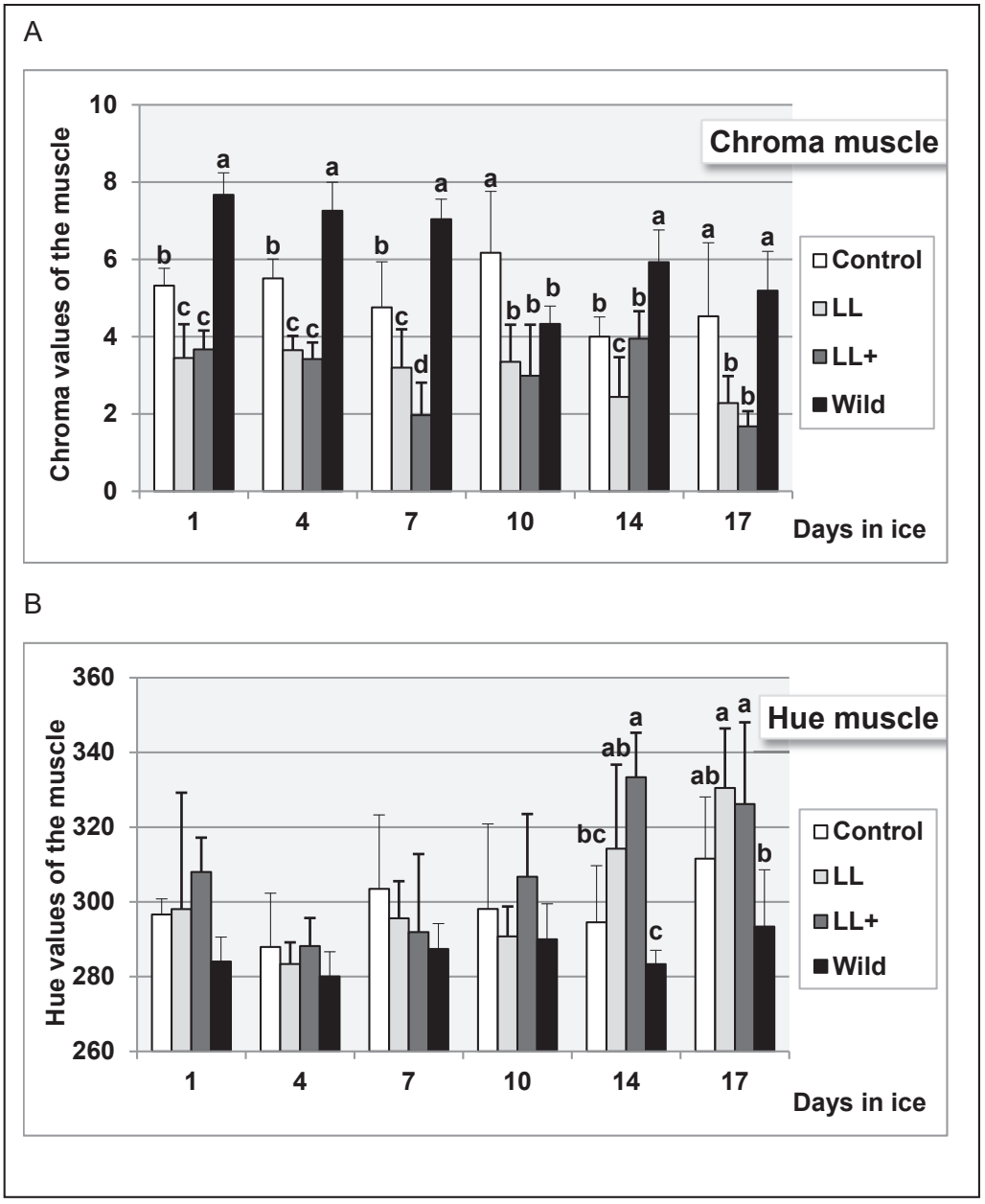

Figure 5. Chroma (A) and Hue values (B) of the muscle of blackspot seabream, wild and fed different diets, throughout ice storage. Different letters in the same day denote statistically significant differences ($P < 0.05$).

3.3.4. Proximate composition and fatty acid profile

On the 1st dph, in the muscle of the blackspot seabream fed the Control and LL+ diets, the protein content was significantly higher than in the other experimental dietary groups. During dph period protein values decreased and the differences between groups found on the 1st dph vanished (Table 3). The lowest lipid content was found in the wild fish group both in the 1st dph and throughout shelf life. Moisture analysis showed

complementary values to those found when studying lipid content, thus high values of moisture correspond to low fat content and vice versa.

Table 3. Proximal composition (g/100 g wet muscle) of the muscle of wild blackspot seabream and blackspot seabream fed different diets, through ice storage. (Mean ± SD).

		Diet			
	Days on ice	**Control**	**LL**	**LL+**	**Wild**
Protein	1	20.48 ± 0.40^{ax}	19.77 ± 0.35^{ay}	20.55 ± 0.46^{ax}	18.99 ± 0.67^{abz}
	4	19.82 ± 0.26^{b}	19.63 ± 0.31^{a}	19.61 ± 0.45^{b}	19.47 ± 0.55^{a}
	7	19.60 ± 0.47^{bx}	19.78 ± 0.44^{ax}	19.98 ± 0.24^{abx}	18.50 ± 0.50^{bcy}
	10	19.36 ± 0.59^{bcx}	19.09 ± 0.33^{bxy}	19.57 ± 0.62^{bx}	18.61 ± 0.24^{bcy}
	14	18.68 ± 0.45^{dy}	18.57 ± 0.27^{by}	19.40 ± 0.35^{bx}	18.17 ± 0.58^{by}
	17	18.81 ± 0.42^{cd}	18.70 ± 0.62^{b}	18.63 ± 0.46^{c}	18.67 ± 0.67^{bc}
Lipid	1	2.99 ± 0.09^{x}	3.96 ± 0.81^{x}	3.60 ± 0.77^{x}	0.91 ± 0.09^{y}
	4	3.33 ± 0.65^{x}	3.91 ± 0.51^{x}	4.15 ± 1.25^{x}	1.45 ± 0.81^{y}
	7	3.45 ± 0.63^{x}	3.87 ± 0.80^{x}	3.78 ± 1.21^{x}	1.67 ± 0.98^{y}
	10	3.66 ± 1.49^{x}	4.54 ± 0.81^{x}	3.96 ± 1.03^{x}	1.74 ± 1.34^{y}
	14	3.67 ± 1.16^{x}	4.80 ± 0.83^{x}	3.84 ± 0.92^{x}	1.45 ± 0.65^{y}
	17	3.81 ± 0.78^{x}	4.77 ± 0.77^{x}	3.39 ± 0.70^{x}	1.39 ± 0.38^{y}
Moisture	1	74.85 ± 0.40^{by}	73.21 ± 0.79^{bz}	75.52 ± 0.96^{by}	78.57 ± 0.76^{x}
	4	75.65 ± 0.47^{by}	74.91 ± 0.82^{aby}	74.67 ± 1.44^{by}	78.55 ± 1.67^{x}
	7	75.63 ± 0.26^{by}	75.03 ± 1.18^{ay}	75.17 ± 1.43^{by}	78.39 ± 2.23^{x}
	10	75.94 ± 1.82^{aby}	74.89 ± 0.71^{aby}	75.29 ± 1.54^{by}	78.89 ± 1.22^{x}
	14	77.25 ± 1.78^{ay}	74.06 ± 1.20^{abz}	75.50 ± 1.04^{bz}	79.55 ± 0.96^{x}
	17	75.78 ± 1.23^{abyz}	74.76 ± 1.99^{abz}	77.24 ± 0.78^{ay}	79.85 ± 0.96^{x}
Ash	1	2.03 ± 0.35^{ax}	1.42 ± 0.04^{ay}	1.44 ± 0.04^{ay}	1.96 ± 0.24^{ax}
	4	1.25 ± 0.07^{by}	1.36 ± 0.12^{ax}	1.30 ± 0.06^{bxy}	1.23 ± 0.05^{by}
	7	1.16 ± 0.11^{bz}	1.44 ± 0.06^{ax}	1.29 ± 0.09^{by}	1.10 ± 0.09^{bcz}
	10	1.17 ± 0.06^{byz}	1.45 ± 0.10^{ax}	1.21 ± 0.07^{by}	1.08 ± 0.09^{bcz}
	14	1.13 ± 0.09^{b}	1.13 ± 0.18^{b}	1.08 ± 0.08^{c}	1.03 ± 0.06^{c}
	17	1.08 ± 0.12^{bx}	0.90 ± 0.07^{cy}	1.02 ± 0.09^{cxy}	0.95 ± 0.11^{cxy}

Different letters in the same line (x,y,z) denote statistically significant differences ($P < 0.05$). Different letters in the same row for each parameter (a,b,c) denote statistically significant differences ($P < 0.05$).

Analysis of the SFA in the muscle of the farmed blackspot seabream showed that on the 1st dph the content of palmitic acid (16:0), the most abundant SFA, was higher in the muscle of the fish fed the LL+ diet (Table 4). 16:0 amounts influenced the total content of SFA both in the muscle and in the diets. Total saturated fatty acid value in the muscle of the wild fish on the 1st dph was significantly lower than that found as a result of feeding

experimental diets (Table 4). The effect of time-on-ice for muscle content of SFA was different depending on the tested diets (Figure 6A).

Table 4. Fatty acid content (g/100 g of fatty acids) of the diets and in the muscle of blackspot seabream, wild and fed different diets on the 1st day post-harvest (Mean ± SD).

Fatty acid	Control		LL		LL+		Wild
	Diet	Muscle	Diet	Muscle	Diet	Muscle	Muscle
14:0	4,40	3.68±0.25^{a}	3,36	3.58±0.15^{a}	5,38	3.38±0.18^{a}	1.55±0.35^{b}
16:0	14,56	18.39±0.18^{b}	17,86	18.13±0.53^{b}	24,83	19.65±1.21^{a}	18.30±0.65^{b}
18:0	3,73	6.34±0.21^{ab}	4,39	6.05±0.24^{b}	6,46	6.68±0.29^{a}	6.46±0.33^{a}
Saturated	24,45	29.58±0.67^{ab}	26,72	28.72±0.65^{b}	37,27	30.64±1.53^{a}	27.46±0.45^{c}
18:1*n*-9	23,84	20.34±1.29^{a}	21,39	19.97±1.43^{a}	19,24	18.91±1.07^{a}	9.10±1.69^{b}
Monoenes	33,91	31.84±1.93^{a}	34,58	33.09±1.20^{a}	37,80	31.74±1.32^{a}	16.10±2.57^{b}
18:2*n*-6	16,57	13.09±0.45^{a}	15,85	9.67±0.84^{b}	10,09	8.19±0.69^{c}	1.27±0.57^{d}
20:4*n*-6	0,59	0.76±0.08^{c}	0,72	0.91±0.10^{bc}	0,64	1.10±0.09^{b}	3.72±0.27^{a}
Σ*n*-6	17,70	15.74±0.53^{a}	17,26	12.12±0.75^{b}	11,49	11.04±0.74^{c}	9.86±0.66^{d}
18:3*n*-3	3,63	1.14±0.05^{a}	2,71	1.19±0.10^{a}	1,34	0.96±0.09^{b}	0.21±0.09^{c}
20:5*n*-3	9,99	5.11±0.53^{b}	5,48	5.90±0.33^{a}	3,13	5.79±0.43^{a}	3.39±0.31^{c}
22:6*n*-3	4,55	8.24±1.33^{c}	8,36	11.35±0.96^{b}	3,91	12.63±1.30^{b}	37.52±2.95^{a}
Σ*n*-3	22,56	18.95±2.32^{c}	19,85	24.15±1.13^{b}	10,63	25.65±1.63^{b}	44.92±2.53^{a}
***n*-3/*n*-6**	1,27	1.20±0.13^{c}	1.15	2.00±0.21^{b}	0.92	2.24±0.25^{b}	4.57±0.45^{a}
Σ*n*-3 LC-PUFA	16,35	16.98±2.24^{c}	15,55	21.73±1.33^{b}	8,08	22.61±1.67^{b}	44.00±2.62^{a}

Different letters in the same line denote statistically significant differences ($P < 0.05$) between muscle content of fatty acids.

MUFA were mainly represented by oleic acid (OA, 18:1*n*-9). On the 1st dph, both the OA and the total MUFA contents in the muscle of the fish fed experimental diets were lower than those contained in the experimental diets (Table 4). Throughout the shelf life, the variations of OA and MUFA content were found to be minor (Figure 6B). In the wild fish muscle, the MUFA values were significantly lower than in farmed fish and during the dph MUFA content increased, especially on the 7th dph (Figure 6B).

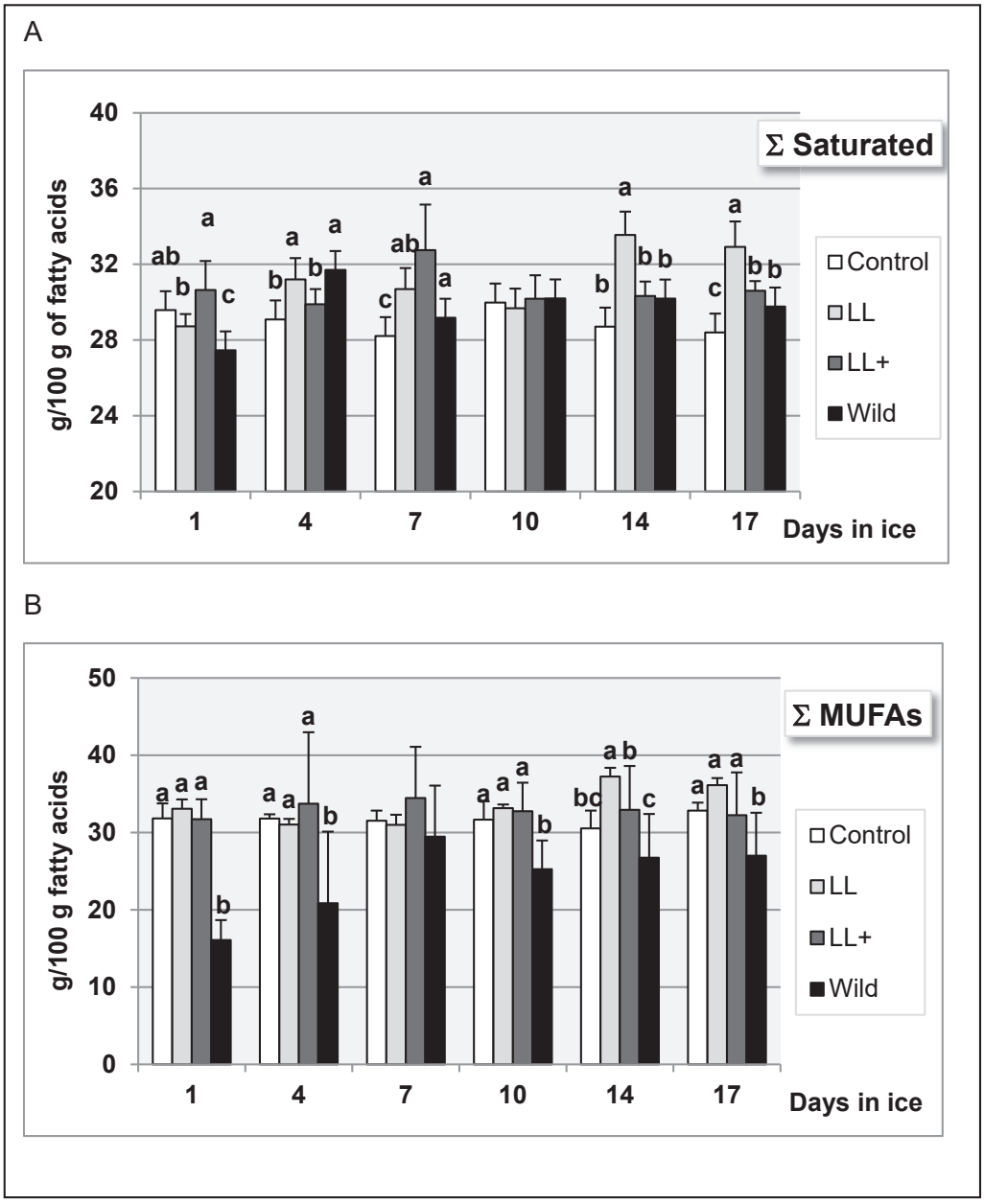

Figure 6. Saturated (A) and monounsaturated (B) fatty acid content (g/100 g of fatty acids) in the muscle of blackspot seabream wild and fed different diets throughout ice storage. Different letters in the same day denote statistically significant differences ($P < 0.05$).

The level of linoleic acid (LA, 18:2*n*-6) found in the muscle of the fish fed experimental diets on the 1st dph was lower than that provided in the corresponding diets (Table 4). The highest value of LA resulted from feeding the Control diet. Wild fish linoleic acid values were about tenfold lower than in fish fed experimental diets (Table 4). During the dph the values were stable (Figure 7A). Similarly, on the 1st dph the values of alpha linolenic acid (ALA, 18:3*n*-3) were lower than those provided in the diet (Table 4). The

highest values registered on the muscle were when fed the Control and LL diets. Wild fish ALA levels were about fivefold lower than for those fed experimental diets. Throughout the dph period the values were stable (Figure 7B) except in wild fish whose values increased significantly (P = 0.005) throughout shelf life.

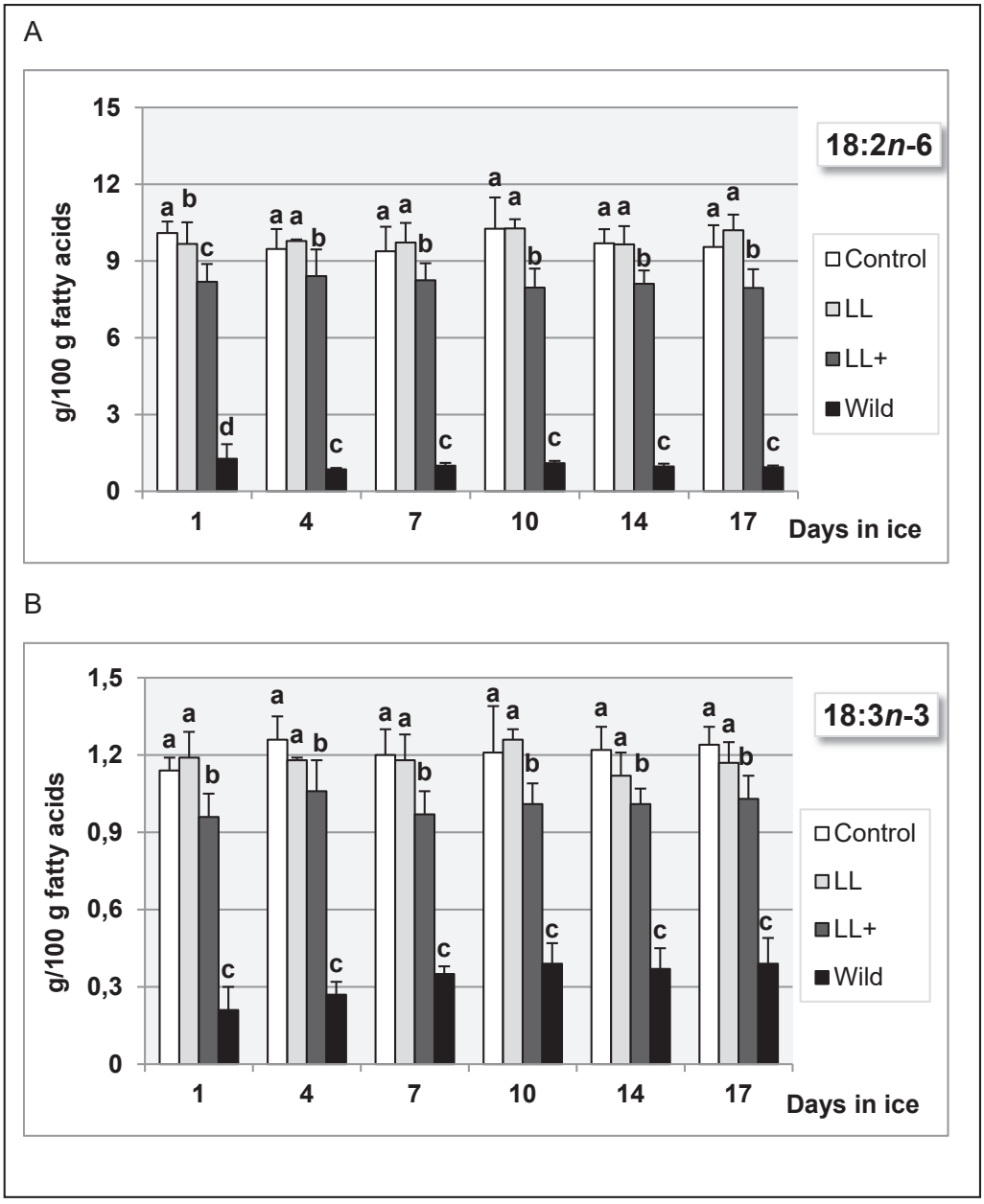

Figure 7. Linoleic acid, 18:2*n*-6 (A) and linolenic acid, 18:3*n*-3 (B) contents (g/100 g of fatty acds) in the muscle of blackspot seabream wild and fed different diets throughout ice storage. Different letters in the same day denote statistically significant differences (P < 0.05).

The levels of arachidonic acid (ARA, 20:4*n*-6) in the muscle on the 1st dph were higher than those provided by the diets (Table 4). Levels in the muscle of the fish fed LL+ diet

were significantly higher compared to values in fish fed control diet. . However, the amount of ARA found in the wild fish muscle was three- to four-fold higher than that found when fed the experimental diets. The variations recorded during the dph showed no clear tendency and the differences found on the 1st day dph were not maintained (Figure 8A). The levels of eicosapentanoic acid (EPA, 20:5*n*-3) were higher in the muscle on the 1st dph than those in the diets except when fed the Control diet (Table 4). The value of EPA in the muscle of wild fish was found to be lower than in experimental diets. During dph period no clear tendency was identified in relation to diets (Figure 8B) but in wild fish the increase of EPA level in muscle was statistically significant ($P = 0.012$, data not shown). The levels of docosapentanoic acid (DHA, 22:6*n*-3) in the muscle on the 1st dph were higher than those provided in the diets (Table 4). The amounts when fed the LL and LL+ diets were significantly higher than when fed the Control diet. As for experimental groups, levels in wild fish were 4-8fold higher than in fish fed experimental diets. As in EPA, the shelf life variations showed no clear tendency (Figure 8C). DHA levels in the muscle of wild fish decreased significantly during the dph period ($P < 0.000$).

The content of total *n*-3 fatty acids and total *n*-3 highly unsaturated fatty acids (*n*−3 LC-PUFA) showed a similar trend to DHA (Figure 9A), the main component. The *n*-3/*n*-6 ratio calculated from the fatty acid content data on the muscle of blackspot seabream indicated highest values than those calculated from the fatty acids content of experimental diets, except feeding the Control diet (Table 4). The values of total *n*-6 in the muscle on the 1st dph were lower than those in the experimental diets, those from the Control dietary group being the highest (Table 4). Wild fish *n*-6 values were significantly lower than in farmed blackspot seabream. During the shelf life the values remained stable (Figure 9B). The *n*-3/*n*-6 ratio calculated in wild fish was higher than in the experimental diets on the 1st dph and throughout shelf life (Figure 9C).

The amount of polar lipids (Figure 10) in the muscle of wild fish was significantly higher than when fed the Control and LL diets but not different in the LL+ diet. Polar lipid levels decreased throughout shelf life (Figure 10), although this was not statistically significant. On the contrary, neutral lipids increased throughout shelf life complementing the findings on polar lipids (results not shown).

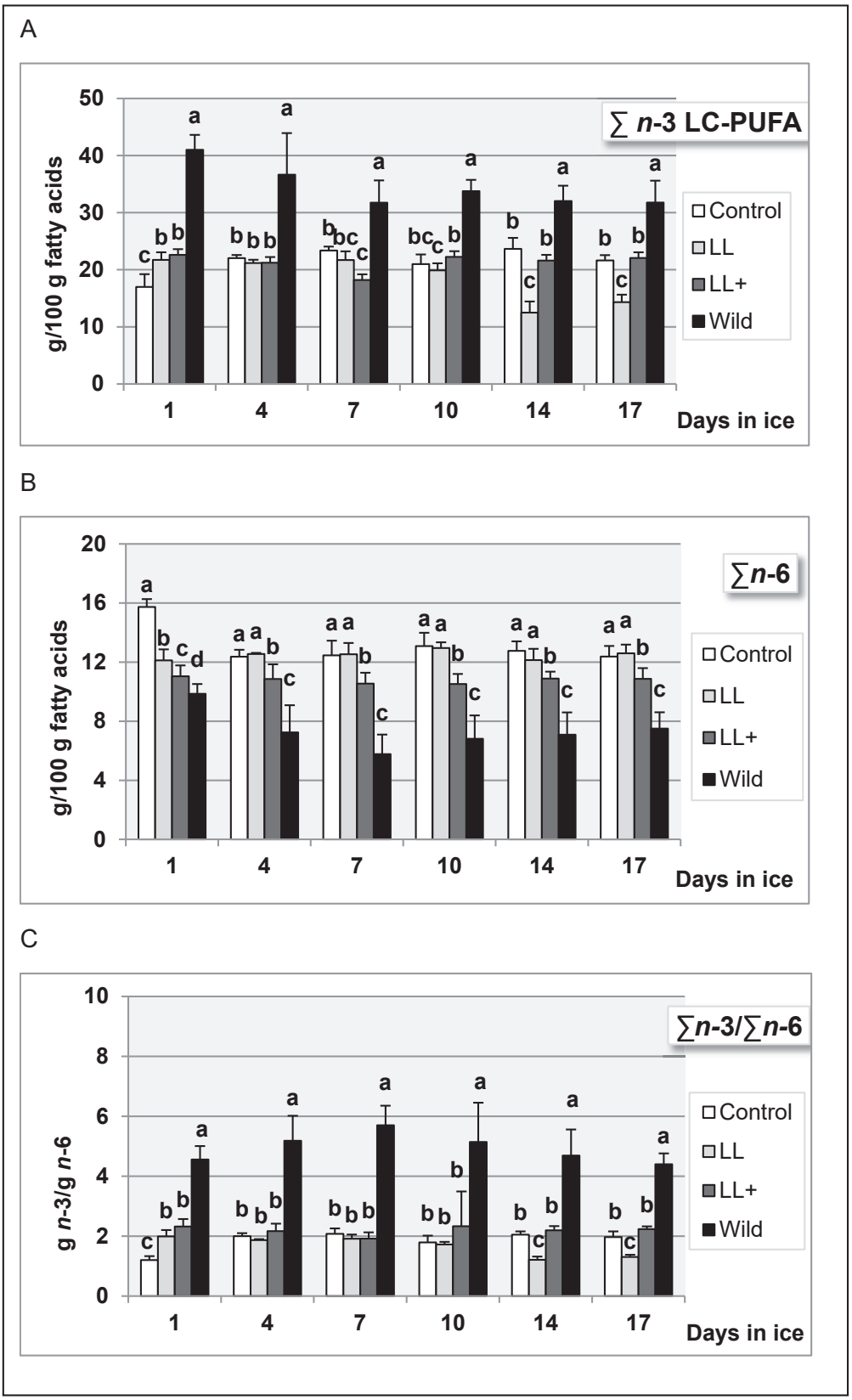

Figure 8. Arachidonic acid, 20:4*n*-6 (A), eicosapentanoic acid, 20:5*n*-3 (B) and docosapentanoic acid, 22:6*n*-3 (C) contents (g/100 g of fatty acds) in the muscle of blackspot seabream wild and fed different diets throughout ice storage. Different letters in the same day denote statistically significant differences ($P < 0.05$).

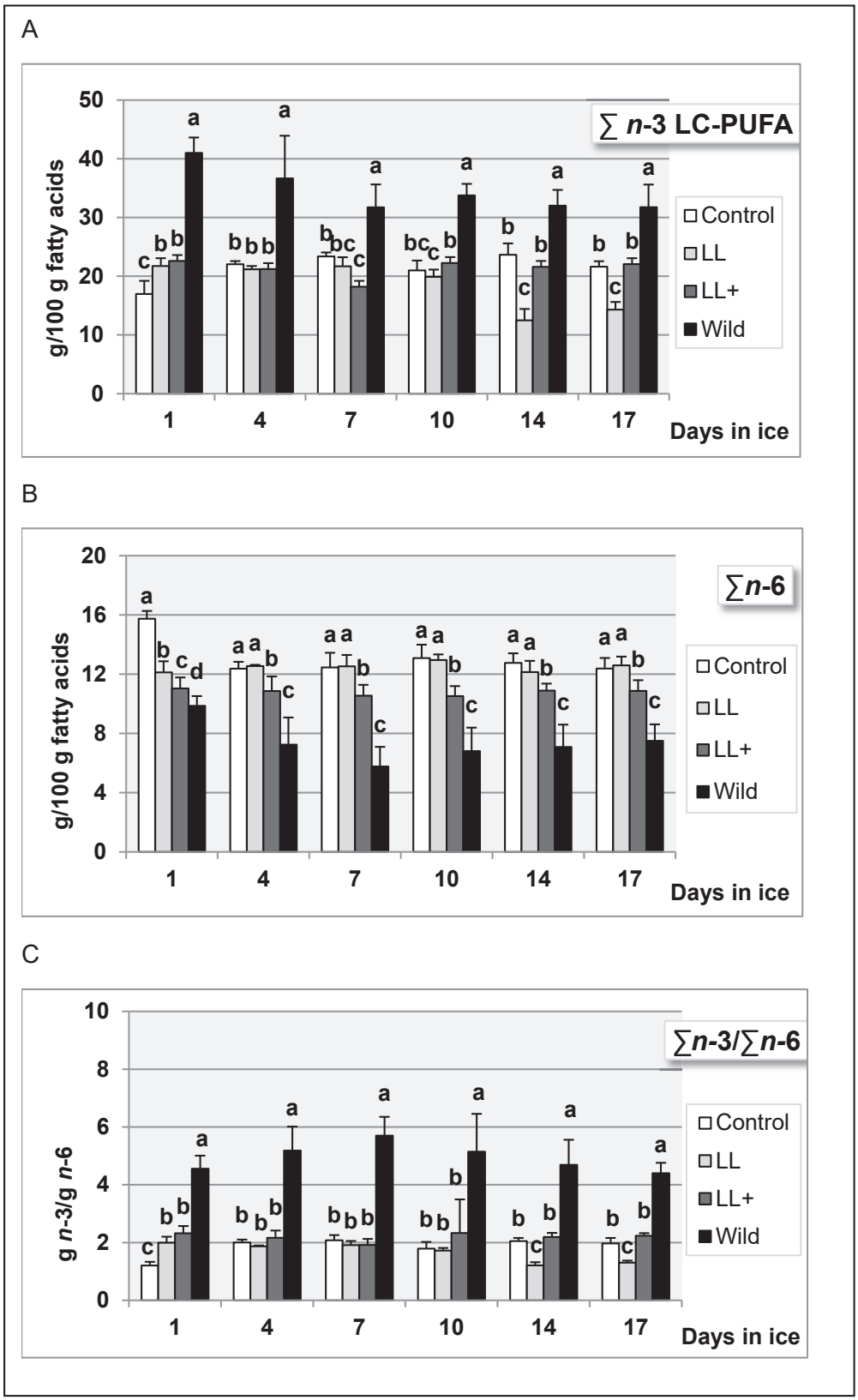

Figure 9. Total *n*-3 highly unsaturated fatty acids (A), total *n*-6 fatty acids (B) and *n*-3/*n*-6 ratio (C) in the muscle of blackspot seabream wild and fed different diets throughout ice storage. Different letters in the same day denote statistically significant differences ($P < 0.05$).

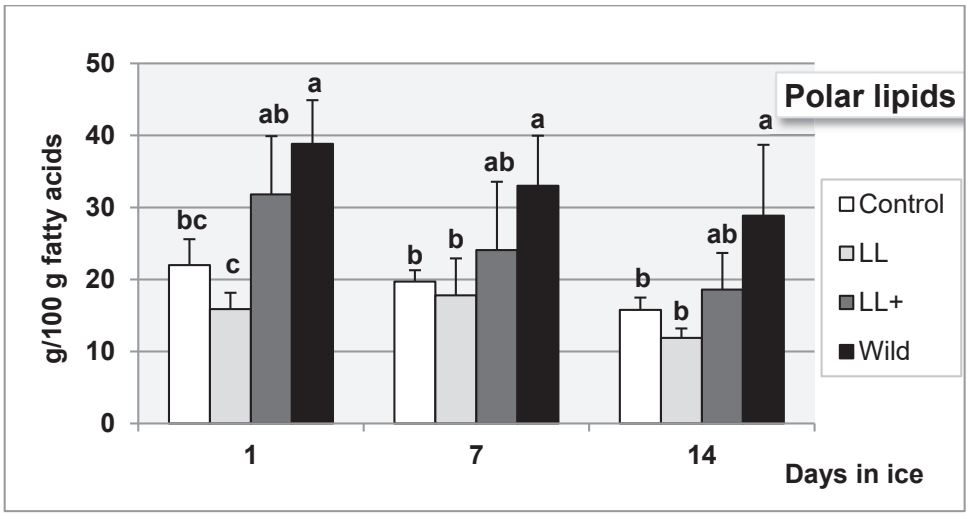

Figure 10. Polar lipid content (g/100 g of total lipids) in the muscle of blackspot seabream wild and fed different diets throughout ice storage. Different letters in the same day denote statistically significant differences ($P < 0.05$).

3.4. Discussion

3.4.1. Muscle lipid distribution.

The tissues were not fixed during frozen sectioning, thus, the observations of lipid storage in the sections excluded those areas with obvious lipid spreading. A significantly higher level of lipid was stored in slow-twitch fibers compared to fast-twitch fibers, with the myoseptum as a major site of adipocyte accumulation in the sections, in agreement with the results observed in Atlantic salmon (Zhou *et al*., 1996). The presence of both intra- and inter-cellular lipids visualized by light microscopy as well as the high proportion of myosepta are reasons for the higher concentration of lipid in slow-twitch fibers (Zhou *et al*., 1995; 1996) suggesting higher intracellular triglyceride storage. Additionally, the red slow twitch muscle fibers have a higher fatty acid β-oxidation capacity, mainly mitochondrial, than the white muscle fibers (Frøyland *et al*., 2000, Stubhaug *et al*., 2005). This is reflected in the increased storage and availability of the fatty acid substrates in the slow- muscle fibers (Nanton *et al*., 2007). Similar to these results are higher levels of histologically observed mitochondrial-associated intracellular lipids, in the slow- compared to the fast-twitch muscle fibers in a survey of various fish species (Shindo *et al*., 1986).

Regarding regional differences, similar results were found upon analysis of Murray cod (*Maccullochella peelii peelii*) muscle (Palmeri *et al*., 2007), with higher lipid amounts in cranial sections. However, fat distribution in the fillet varies within species, as shown by ElMasry and Wold (2008) when they analyzed the fat distribution in the muscle of six

different fish species. Lean fish such as the Atlantic halibut or catfish (*Ictalurus punctatus*) showed higher lipid concentrations in the muscle fibers close to the head. Fatty species such as herring or mackerel showed a regular distribution. In salmon, Katikou *et al*. (2001) found higher lipid concentrations in the cranial sections, also with differences between the dorsal and ventral areas. Álvarez *et al*. (2009 a, b) compared the lipid composition of white muscle in different areas of wild and farmed blackspot seabream, and found that the dorsal portion of the fillet has a higher proportion of triacylglycerol accumulation in both the farmed and the wild fish. This large amount of lipids prevented us from being able to distinguish differences based on diets when studying the cranial area. In the medial and caudal areas, however, and as was shown in different studies based on vegetable ingredients (Izquierdo *et al*., 2005; Montero *et al*., 2008; Castro *et al*., 2010, 2015) diet formulation produces differences in lipid deposition, in this case by adding fish meal to the LL+ diet.

3.4.2. Sensory evaluation

On comparing wild and farmed fish, the attribution of some demerit points related to colour and brightness of the skin was challenging. Luminosity is comparable in wild and farmed fish. Redness in the skin or in the fins, however, is clearly more intense in wild fish and these differences need further development in the scheme. An advantage of the QIM arises from the fact that the maximum negative score corresponds to the end of the shelf life of the samples, so demerit points act in this way as a reference to predict suitable storage time in ice (Sveinsdóttir *et al*., 2009). The scheme developed by Sant'Ana *et al*., (2011) for wild blackspot seabream involves 14 parameters with 30 available demerit points. A simplification of the scheme with a maximum of 12-14 demerit points, corresponding with the maximum shelf life of the species, similar to that developed for the gilthead seabream (Huidobro *et al*., 2000), but including farmed blackspot seabream particularities such as colour, could be practical.

Throughout shelf life when the QIM was applied, wild fish obtained higher scores, suggesting a further decay motivated presumably by stress in the capture and different microbiological flora as it was suggested in sea bass (Castro *et al*., 2006) or in gilthead seabream (Alasalvar *et al*., 2002a) when comparing wild and farmed samples. In the present study, although wild fish was obtained through traditional extractive fishing, it was kept in ice after capture and throughout transport and delivery. This fact could minimize the effect of this different handling comparing both wild and farmed fish. On the other hand, Alasalvar *et al*. (2002a) consider that the higher proportion of highly

unsaturated fatty acids in the wild gilthead seabream might fuel spoilage. In this sense, our results confirm that *n*-3 fatty acid group has a positive statistical correlation with spoilage and on the contrary fat content, SFA, MUFA and *n*-6 are related to a delayed spoilage especially with a prolonged dph period. In halibut (*Hippoglossus hippoglossus*) Guillerm-Regost *et al*. (2006) did not find a correlation between diet and shelf life, however the differences in fat, SFA, MUFA, PUFA and *n*-3 in the muscle were not as wide as those between wild and farmed blackspot seabream.

3.4.3. pH and colour

The pH values were higher in the muscle of wild fish than in their farmed counterparts, as also recorded in cod (*Gadus morhua*) (Kristoffersen *et al*., 2006; Olsson *et al*., 2007), halibut (Olsson *et al*., 2003) and gilthead seabream (Attouchi and Sadok, 2010). The acidosis in the fish muscle after sacrifice is produced by accumulation of protons subsequent to the ATP breakdown in anaerobiosis (Robergs *et al*., 2004) being the energetic reserves, more extensive in farmed than in wild fish (Kristoffersen *et al*., 2006; Castro *et al*., 2015). In addition, the loss of these reserves is less extensive than in wild fish owing to the stress that comes from fishing capture (Lowe *et al*., 1993). In farmed fish, the inclusion of different ingredients in the diets did not produce significant changes in muscle pH values (Menoyo *et al*., 2004; Castro *et al*., 2015). The subsequent increase of the pH values arose from bacterial basic compounds resulting from fish muscle spoilage and the increase was analogous in all experimental groups. The negative correlation found between pH and lipid fractions depends on the dietary background of the fish, wild or farmed, as well as their muscular composition, as was observed in lean fish species where higher pH values are found with less muscle protein content (Ruff *et al*., 2002).

In farmed fish, lightness depends on the surrounding environmental luminosity during the growing period (Ginés *et al*., 2004) and population density in the cages (Van der Salm *et al*., 2004). In the present study, the farmed blackspot seabream were reared in marine cages at low density and thus in a background environment lighter than that of wild fish. Consequently, cage farming conditions would produce L* skin values lower than in their wild counterparts. In any case, the differences disappear during the dph period ,showing a lightening of the skin (Pavlidis *et al*., 2006; Álvarez *et al*., 2012).

The intensity of red and yellow is conditioned by diet, as it has been established in red porgy (García-Romero *et al*., 2010, Kalinowsky *et al*., 2005, 2011), a fish species with a characteristic colour akin to the blackspot seabream. Thus, the highest proportion of red

pigments in the skin of the wild fish would correspond to higher values of a* that remain constant during the ice shelf life. Yellow pigment amounts, the basis for b* assessment, seem to have produced values comparable in all experimental groups. However, the spoilage during shelf life had a higher influence on the b* values of farmed fish. This means that the same degree of colour saturation in the fresh caught fish in all experimental groups produces a darker farmed blackspot seabream during the dph period. This pattern of post-mortem changes in the skin chromaticity can be associated with changes in the intensity of the pigments within skin chomatophores (Pavlidis *et al*., 2006). As for the Hue, in farmed fish, the preponderance of the yellowish tones determined in the first dph diminished towards the end of the shelf life, as it was shown in red porgy by Pavlidis *et al*. (2006), and the differences found in farmed fish Hue disappeared.

Although lightness is not affected by the protein source in farmed fish (Parisi *et al.*, 2004), the colour of fillets is influenced by the oil source and level inclusion (Turchini *et al*., 2013b). The level of inclusion of vegetable oils in the diet affects the redness and yellowness in species with low levels of colour saturation in muscle such as the gilthead seabream, even when the inclusion does not affect total lipid amounts in the muscle (Menoyo *et al*., 2004). In the present study a considerable variation in the fat level in the muscle of the farmed fish was found, corresponding to a greater presence of MUFA and lower *n*−3 LC-PUFA, thereby promoting variations in the lightness (Turchini *et al*., 2013a) and also a more yellowish tone in the muscle of the farmed fish. Throughout the dph , the b* values in the muscle of wild blackspot seabream were close to those in farmed fish, probably due to the slight decrease in the proportion of MUFA. Despite this small variation in the yellowness, the value of Hue was unaltered since the value of the redness was close to zero, as described in gilthead seabream (Álvarez *et al*., 2012). Conversely, saturation values were affected in the wild blackspot seabream, which decreased throughout shelf life. However, these values were always above those of farmed fish.

The higher pH values along with a smaller proportion of protein in the muscle of wild blackspot seabream conditioned the greater brightness. As time progresses during the dph period, the pH increased in all experimental groups, together with a decline in the proportion of protein which promotes a lighter appearance of the muscle. In wild blackspot seabream, high levels of *n*-3 had an indirect effect on the brightness of the muscle due to its influence on pH values leading to the higher L* values during the dph period compared to farmed. Variations of pH accompanying L* changes were described in gilthead seabream when studying the effect of starvation (Ginés *et al.,* 2002).

3.4.4. Proximate composition and fatty acid profile

The total fat content in the wild blackspot seabream was twofold lower than in the farmed fish, showing that farmed fish are capable of greatly increasing their muscle lipid proportion when fed high-energy diets. In this sense, the protein/lipid ratio in the diet could modify the whole body fat content (Silva *et al*., 2006). Figueiredo-Silva *et al*. (2010) studied this protein/lipid ratio in blackspot seabream diets including also, as a source of variation, the protein origin. Accordingly, different proportions of fish meal and vegetable ingredients were included in the experimental diets which produced, at juvenile size, a significant effect on the fat amount. However, in the present study and specifically focusing on the biochemical composition of the muscle, the differences based on protein/lipid composition or protein source, disappear at commercial size. This means that fish metabolism in lean species shows a poor utilisation of dietary lipids (Valente *et al*., 2011), accumulating similar fat amounts in the muscle when fed diets with different lipid content or composition, either from fish or vegetable sources. Additionally, the deposition of lipids from dietary protein could increase due to higher ratio protein/energy (Francis and Turchini, 2017) in LL and LL+ diets.

Both protein and moisture contents decrease during shelf-life. The protein reduction found during shelf-life could be the result of the dripping process caused by protein denaturation, as it was reported in farmed turbot with a decrease in sarcoplasmic protein content during shelf life (Aubourg *et al*., 2005). The moisture content in the fillet decreased during shelf-life in farmed fish but this decrease was not linked to the fat content, as previously described (Castro *et al*., 2010). It was linked, however, to protein content and the dripping process.

Dietary fatty acids are found as storage fat, located in adipose tissue rich in triglycerides, and structural lipids consisting of phosphoglycerides and cholesteryl esters, which are integral parts of biomembranes and usually are rich in polyunsaturated fatty acids (Tocher, 2003). Polar lipids belong to the structural lipids fraction which is characterized by long chain polyunsaturated fatty acids including EPA and DHA (Tocher, 2003), characteristic of wild fish. Neutral lipids, including triglycerides, are the main component of lipid depots, larger in the farmed fish than the lean wild fish. The lipid fraction of fish tissues is most significantly affected by the nature of dietary lipids (vegetable oils versus fish oils), with triglycerides being more affected than phospholipids (Figueiredo-Silva *et al*., 2005). Similar distribution was found in a lean species as turbot (Regost, 2003). The essential fatty acids DHA and EPA, however, were incorporated at high concentrations

in the polar lipid of the muscle, which is a characteristic pattern of the fatty acid incorporation in fish muscle (Nanton *et al*., 2005).

The values of SFA accumulated in the muscle were found to be comparable, independently of the amount supplied by diets, whether low as in Control or high as in LL+. This implies that determined SFA levels are essential for the fish metabolism and suggest a minimum selective storage of these fatty acids (Izquierdo *et al*., 2003). In the present study, MUFA in the diets constitutes 30% or more of the total lipid content, and the values of OA and MUFA accumulated in the muscle were similar to or lower than that of the diet. When the amounts provided in the diet are less than 30% MUFA, a relatively higher proportion accumulates in the fillet (Palmegiano *et al*., 2007; Figueiredo-Silva *et al*., 2010). This can be understood, as the amount of OA and MUFA appear to be stable independent of the inclusion level or the ingredients source and there is a basal requirement regardless of the input. However, the MUFA profile in the muscle of the wild fish is inferior and that means that the excess of energy from the diet is stored as MUFA and to a lesser extent as SFA, probably due to a higher efficiency in the β-oxidation of SFA for energy production (Francis and Turchini, 2017), affected more by changes in energy demand that dietary fatty acids (Stubhaug *et al*., 2007).

Different studies on the gilthead seabream or sea bass have shown that feeding diets with increasingly vegetable-based ingredients increases the amount of LA and ALA in the fish fillet (Izquierdo *et al*., 2005, Montero *et al*., 2008, Castro *et al*., 2010, 2015). In the present study, independently of vegetable oil source, diets LL and LL+ with low lipid levels promoted differences in LA and *n*-6 compared to the high lipid content of the Control diet, as it was previously described in another lean fish as sole (*Solea solea*) (Rueda-Jasso *et al*., 2004). With juvenile samples of blackspot seabream, different results were found depending on the nature and proportion of vegetable ingredients. However, the differences were not as extensive as those found in commercial sized samples (Silva *et al*., 2006; Palmegiano *et al*., 2007).

Muscle percentages of ARA, EPA and DHA were mostly higher than the amounts contributed by the experimental diets. It was especially noticeable when comparing the LL+ and LL diets. The selective accumulation of DHA in blackspot seabream produced a similar deposition of DHA in the muscle in spite of the minor proportion of DHA in the LL diet. In juvenile blackspot seabream (Figueiredo-Silva *et al*., 2006; Palmegiano *et al*., 2007), these fatty acids are found in significantly lower proportions in the muscle, especially for EPA. The authors suggest that in this age range EPA is more efficiently catabolized than DHA and that DHA might have a higher biological value than EPA

during the growing stage. Besides, retention efficiency of DHA could be higher than for EPA (Sissener *et al*., 2016). A shorter farming period and consequently less time of exposure to the diets may have an effect on these results. Álvarez *et al*. (2009b), studying fully grown specimens, found that as in the present study EPA values were higher in farmed fish than wild. In commercial sized fish, when growing requirements decline, *n*−3 LC-PUFA requirements decrease and blackspot seabream are able to accumulate these high quality fatty acids independent of diet supplies. This fact is especially important from the nutritional point of view and gives an extra incentive to the consumer of the commercial sized blackspot seabream.

Total *n*-3 and *n*−3 LC-PUFA acts as a measure of the benefits of fatty acid from fish muscle. On feeding with LL and LL+ diets, the values of these quality indicators were improved compared to feeding with the Control diet. Although this can be achieved by a careful formulation based on a fish oil balanced contribution as suggested by Figueiredo-Silva *et al*. (2006), a better retention of *n*-3 LC-PUFA has been shown over the grow-out period when fish are fed diets rich in SFA and MUFA (Francis *et al*., 2014), as it happens specially feeding LL+ diet. Otherwise, the increase in vegetable ingredients reduces *n*-3 proportion significantly (Palmegiano *et al*., 2007). The experimental diets achieved remarkable *n*-3 values without unduly increasing *n*-6 amount, which is one of the more important challenges when feeding vegetable ingredients. Thus, the *n*-3/*n*-6 ratio, although inferior to that of wild fish, can be considered suitable and similar to related aquaculture species such as the gilthead seabream (Castro *et al*., 2015; Grigorakis *et al*., 2002).

Yildiz *et al*. (2016) did not find variations in the fatty acid composition of rainbow trout fillets during a dph period of 12 days, while Alexi *et al*. (2017) found significant shelf-life variations in gilthead seabream both for SFA and MUFA. In our case, the fatty acid profile showed a different spoilage progress depending on the experimental group. Farmed fish showed analogous variations per 100 g of fat in the muscle independently of the experimental diet. For example, on studying the effects of different diets on SFA in other farmed species such as the gilthead seabream or sea bass, SFA were stable during the dph period (Pirini *et al*., 2000; Castro *et al*., 2010; 2015). However, in wild blackspot seabream, an increase in SFA was shown, as was also described in other wild species such as the sardine (*Sardinella gibbosa*) (Chaijan *et al*., 2006).

The recorded increases are even higher when looking at MUFA. From the first week of storage until the 17th day in ice the percentage of MUFA in the muscle of the wild blackspot seabream increased almost twofold. This increase determined during the dph

period was produced by the depletion of longer fatty acids and the release of these fatty acids from cellular structures by means of degradation enzymes (Castro *et al*., 2010).

The rest of fatty acid fractions showed less noticeable but statistically significant variations. These changes follow a similar pattern with scarce variations during the shelf life in farmed and increases in the wild blackspot seabream, such as for ALA and EPA. DHA does not follow the mentioned dynamic as the high values of 30% determined during the first week of storage were reduced during the dph period. In spite of this reduction that impacted the *n*-3 fraction, the *n*-3/*n*-6 ratio was maintained favorable during the studied shelf life. Thus, the cardiovascular protection and health beneficial properties associated with fish consumption can be achieved with blackspot seabream fed low fat diets. 100 g of farmed fish fillet provides a higher amount of *n*−3 LC-PUFA than 100 g of wild fish and this is possible due to the higher proportion of fat in the muscle that compensates for the lower proportion of specific fatty acids. This has been described in other lean farmed species such as sole (Hossain, 2011), but the particularity in blackspot seabream is the substantial proportion of DHA in muscle fat of wild individuals.

It has been suggested that current diets in farmed fish rich in vegetable ingredients are adequate to sustain maximum growth and optimum health but they are not sufficient to maintain n−3 LC-PUFA levels similar to those in wild fish muscle (Tocher, 2015). The use of low fat diets during marketable size production of blackspot seabream was effective in obtaining farmed products rich in long-chain n-3 PUFAs and supplied sufficient n−3 LC-PUFA even exceeding the biological requirements of the fish. In any case, preserving the n-3 polyunsaturated fatty acid content of the final fishery product has to be an additional consideration when designing the diets (Hunter and Roberts, 2000). In the present study, the LL+ diet maintained a higher level of n-3 throughout shelf life than the LL diet. Additionally, diet LL+ presented a lower level of n-6, hence a better n-3/n-6 balance, which is nutritionally preferable from the consumer point of view.

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CAPÍTULO 4. Texture changes during chilled storage of wild and farmed blackspot seabream (*Pagellus bogaraveo*) fed different diets

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Abstract

The impact of changes in dietary lipids and protein sources on texture was evaluated on farmed blackspot seabream (*Pagellus bogaraveo*) throughout 14 days of ice storage and compared with wild fish. A commercial diet formulated with a high proportion of lipids, and two diets formulated with an important reduction of lipid levels by 60% and adding either plant protein sources (LL diet) or fishmeal (LL+ diet), were supplied during growth until commercial size was attained. In the wild fish, the raw fillet hardness was significantly higher than in farmed fish during the entire ice storage period. In the farmed fish, an increase of muscle lipid accumulation and change of fibre density were responsible for the variations in texture in the raw fillet. The highest reduction was found in fish fed with diets LL+ and LL. The texture parameters studied on the cooked fillets showed no significant differences, neither attributable to the diets nor to the ice storage period.

4.1. Introduction

The blackspot seabream (*Pagellus bogaraveo*) is a fish species with an elevated commercial value, thanks to its flesh firmness and flavour. The aquaculture production of blackspot seabream has become a promising market alternative considering that this semi-pelagic marine fish, commonly found off the European coasts, is being overfished and causing the general collapse of traditional fisheries (Lorance, 2011; Pinho et al., 2014). Hence, captures have decreased from 8910 tonnes in 1980 to 1385 tonnes in 2018 (FAO, 2021). Nevertheless, fish farming promotes compositional variations that affect flesh quality (Izquierdo et al., 2003). Diet ingredients and a constant supply of food result in large deposits of lipids, as previously reported on different farmed species, such as the gilthead seabream (*Sparus aurata*) (Grigorakis et al., 2002), European seabass (*Dicentrarchux labrax*) (Alasalvar et al., 2002; Fuentes et al., 2010), Atlantic halibut (*Hippoglossus hippoglossus*) (Olsson et al., 2003) or turbot (*Scophthalmus maximus*) (Martinez et al., 2010), especially as compared with their wild counterparts. Regarding diet ingredients, the global demand for fish meal and fish oil has steadily increased the costs of the diets for farmed fish (Tacon et al., 2008; Castro et al., 2015). In this context, blackspot seabream represents an interesting opportunity, as carnivorous species maintain a reasonably good growth potential despite the reduction in the inclusion of fish oil in practical diets (Figueiredo-Silva et al., 2009). This desirable performance is conditioned upon an enhancement of energy available via the inclusion of carbohydrates,

only effective in maintaining growth parameters whilst the protein source comes from fishmeal (Valente et al., 2010).

Regarding quality, texture is one of the most important parameters, not only for producers but also for consumers (Hyldig et al., 2001), since the firmness, changes throughout the shelf life are closely associated with acceptability (Cheng et al., 2014). In farmed fish, the ample lipid content and its distribution influence the texture properties of the flesh (Lie, 2001) that can be modulates through the formulation of diets. Suárez et al. (2010) reported, after a feed restriction, an enhancement in the muscle texture associated with the decrease of lipid deposits in the structural components of the muscle. The muscle structure of farmed fish has been associated with a softer texture than that of wild fish (Periago et al., 2005). Factors of potential relevance in fish farming, such as diet composition and feeding regimes, promote variations during muscle growth in the muscle fibres, as distribution or girth (García de la Serrana et al., 2013). The effect is noticeable in the two main processes: the hyperplastic input of new fibres, known to occur in the skeletal muscle of blackspot seabream (Silva et al., 2009), and the hypertrophic growth of previously formed fibres (Alami-Durante et al., 2010a), resulting from the balance between protein synthesis and degradation. Accordingly, histological studies of fibre recruitment, morphology or distribution are a suitable instrument to understand texture properties of the species under study.

The present work focuses on those changes in the density of the muscular fibres and those texture variations on farmed blackspot seabream fed different diets and compared with their wild counterparts, during chilled storage.

4.2. Materials and methods

4.2.1. Growth trial

FELASA category C endorsements and the European Economic Community animal experimentation guidelines directive of 24 November 1986 (86/609/EEC) and (2010/63/EU) were followed. Blackspot seabream (mean weight of 155.1 ± 30.4 g, 14 months old) from the Oceanographic Spanish Institute (Vigo, Spain), were fed 20 months three diets, Control, LL and LL+. The Control diet (Bes-Power, Sparos, Faro, Portugal) consist on lipids (29.33% dry weight), mostly from fish oil (170 g/kg) and two thirds from plant sources and one third from fishmeal as protein content. To decrease lipid levels by 60% compensating the energy intake by including carbohydrates, two experimental diets

were formulated, LL (low lipid diet) and LL+ (low lipid diet + fish meal). In LL fish oil reduced (50 g/kg) but the proportions of protein sources were maintained. In LL+, fish oil reduction did not vary (50 g/kg) but most of the protein come from fishmeal (456 g/kg in LL+ diet against 216 g/kg in LL diet).

4.2.2. Sample preparation

At the end of the experimental trial, 30 farmed blackspot seabream per diet (405.2 ± 68.6.4 g), were sampled. Furthermore, 30 wild fish (509.0 ± 46.8 g), were obtained upon arrival at Vigo Fish Market (Pontevedra, Spain) within 10 hours of being caught. Fish were packed as whole ungutted fish with flaked ice into polystyrene boxes with holes for drainage ice and shipped to the High Specialization Aquaculture and Biotechnology Service (SABE) (ULPGC, Gran Canaria, Spain), arriving within 24h. Fish were stored at 4°C for 14 days post-harvest (dph). During storage, five fish per diet, randomly chosen, were obtained at 1, 4, 7, 10 and 14 post-harvest day (dph) and individually sampled.

4.2.3. Texture profile analysis

The Texture Profile Analysis (TPA) was made using a TA.XT2 Texture Analyser (Stable Micro Systems Ltd., Surrey, UK). The analysis comprises whole ungutted fish and raw and cooked fillets carried out on six different fish from each dietary group. For whole fish, the compression was made over the lateral line at one cm from the operculum. Two successive cycles with a plunger of 12 mm Ø to mimic the compression applied by a researcher during a sense evaluation (Ginés et al., 2002). Depth 7mm, speed 0.8 mm/s (5 s between cycles). The left fillet was unskinned and then divided in square pieces (2.5x2.5x1.5 cm) collected above the lateral line from cranial, central and caudal location. For fillet texture examination a compression plate (100 mm Ø) at 0.8 mm/s were used, forcing a deformation (60% of the original thickness) (Ginés et al., 2004). Following the same procedure that for raw fillet, three fragments were baked in an air-heated oven (Compact Eurofred, Barcelona, Spain) at 115 ℃, for 10 min in packed in aluminum boxes. The deformation of the original length for cooked fillet was 80%.

4.2.4. Proximate composition

Fish fillets from the right side were homogenized and subjected to proximal analysis by a FoodScan™ (FOSS, Hillerød, Denmark) (AOAC, 2007.04). Dry matter content was calculated by drying in an oven (110ºC) until constant weight, ash content by combustion in a muffle furnace (600ºC for 12h).

4.2.5. Histology. Muscle fibre studies

At the end of the experiment, 15 fish per diet were sampled. Muscle tissue from the medial section, under the lateral line, was fixed in 10% neutral-buffered formalin, dehydrated in an ethanol series and embedded in paraffin wax. Sections of five µm were prepared with a Leica microtome (Leica Instruments GmbH, Hubloch, Germany) and stained with haematoxylin and eosin (Luna, 1968) for histological evaluation. Fibre number among groups was evaluated with an image analysis package (Image-Pro Plus software, Media Cybernetics, Atlanta, GA) attached to a photomicroscope (Olympus CX41, Tokyo, Japan). Three different microphotographs were randomly taken per section (10× objective magnification). To determine the fibre density, three measurements, at separated positions of each image (nine per fish), were recorded and subsequently averaged. Fibre density (fibres mm^{-2}) was calculated as the number of fibres per mm^2 of muscle cross-sectional area (Rincón et al., 2016).

4.2.6. Statistical analysis

Data were submitted to a general linear model with diet and time of storage as fixed factors and body weight as a covariate using a SPSS Statistical Software System 26.0 (Armonk, NY: IBM Corp.). Those significant differences were evaluated by Duncan's multiple range tests. Pearson correlation analysis determined those interactions between biochemical composition and texture parameters.

4.3. Results and discussion

The total fat content was twofold lower in the wild blackspot seabream than in the farmed fish (Table 1), thus showing that farmed fish can increase the proportion of muscle lipid when fed high-energy diets. Studies on blackspot seabream (Silva et al., 2006) have referred to the protein/lipid ratio as a source of variation in the fat content of the whole body. Figueiredo-Silva et al., (2010), in order to study the effect of protein source, formulated some experimental diets with different proportions of fish meal and vegetable ingredients. These inclusions produced, in juvenile fish, a significant impact on the fat content. However, at the commercial size, as in the present study, no differences based on the protein/lipid ratio or the protein sources were recorded. This means that the metabolism of full growth fish from lean species, like blackspot sea bream, shows a poor utilisation of dietary lipids (Valente et al., 2011), accumulating similar fat amounts in the muscle independently of the fed diets either from fish or vegetable sources. The deposition of lipids could increase, though, from dietary protein when the ratio protein/energy increases (Francis et al., 2017) as in the low lipid diets LL and LL+.

Table 1. Proximal composition (g/100 g wet muscle) of the muscle of wild blackspot seabream and blackspot seabream fed different diets (Mean ± SD).

	Diet			
	Control	**LL**	**LL+**	**Wild**
Protein	20.48±0.40^{a}	19.77±0.35^{b}	20.55±0.46^{a}	18.99±0.67^{c}
Lipid	2.99±0.09^{a}	3.96±0.81^{a}	3.60±0.77^{a}	0.91±0.09^{b}
Moisture	74.85±0.40^{b}	73.21±0.79^{c}	75.52±0.96^{b}	78.57±0.76^{a}
Ash	2.03±0.35^{a}	1.42±0.04^{b}	1.44±0.04^{b}	1.96±0.24^{a}

Different letters in the same line denote statistically significant differences ($P < 0.05$).

The influence of fat content in the muscle on the texture of the fillet is shown in Table 2. Texture studies on different fish species have reported a significant loss of hardness, and hence a softening of the flesh associated with the increment of the fat content (Ginés et al., 2004; Ingebrigtsen et al., 2014; Másílko et al., 2015; Menoyo et al., 2004; Thakur et al., 2003; Thakur et al., 2009). This relationship associated with a fattier flesh, even when it is not systematically observed, always leads to a softer texture (Lefevre et al.,

2015). Thus, studying the hardness in whole blackspot seabream, or in the fillet, raw or cooked (Table 2), the muscle fat content was negatively correlated with the maximum force to compression.

Table 2. Pearson's correlation coefficients between chemical composition and texture parameters (hardness and springiness) of whole fish, raw fillet and cooked fillet (Pearson's coefficient and *P* value) (n=200).

	protein	**lipid**	**whole hard**	**whole spring**	**raw hard**	**raw spring**	**cooked hard**
lipid	,204 *,021*						
whole fish hardness	,219 *,014*	-,352 *,000*					
whole fish springiness	,346 *,000*	,094 *,176*	,258 *,005*				
raw fillet hardness	,014 *,444*	-,579 *,000*	,565 *,000*	,285 *,002*			
raw fillet springiness	,540 *,000*	,297 *,001*	,091 *,183*	,216 *,016*	-,208 *,019*		
cooked fillet hardness	,031 *,379*	-,259 *,005*	,152 *,065*	-,005 *,482*	,247 *,007*	,003 *,489*	
cooked fillet springiness	,271 *,003*	,323 *,001*	-,335 *,000*	,026 *,400*	-,363 *,000*	,062 *,271*	-,318 *,001*

The highest influence of fat content was recorded in the raw fillet of the blackspot seabream, explaining more than 30% of the total variation. This value is around threefold higher than that found by Aussanasuwannakul et al. (2011) for rainbow trout (*Oncorhynchus mykiss*), probably based on the ample range of fat content deposited in the muscle of lean species, as blackspot seabream, when comparing farmed with wild fish. In contrast, studying Atlantic salmon (*Salmo salar*) a representative oily fish, Johnston et al. (2006) found no correlation between lipid content and fillet hardness. Despite the significant increase of lipid content from 46 to 84%, comparing wild and farmed salmon, these lipids in the muscle were not able to explain the observed differences in texture. When the farmed fish is fed diets that promote important differences in fat muscle storage, a significant negative correlation between muscle lipid content and flesh hardness could be explained for 50% of the variation (Xu et al., 2016).

In our case, the fat increase was three or four-fold larger and similar to that reported by Fuentes et al. (2010), comparing wild and farmed European sea bass. The total fat content in the muscle was positively correlated in both raw and cooked fillet, studying shape recovery after the first compression, the springiness (Table 2). However, total protein content had a significant role, especially in the raw fillet, being more important than total fat content to determine the springiness and it explained, together with fat content, around a 40% of the total recorded variation.

The values of hardness registered on whole fish were positively correlated with those of raw fillet, despite the effect of skin integrity on the maintenance of muscle structure and the difficulty to assess the texture of whole fish due to the lack of a uniform structure (Hyldig & Nielsen, 2001). Correlation statistics between hardness of raw and cooked fillets were positive and significant but low (Table 2). It has been described that after cooking, the effect of muscle fat content on the mechanical resistance of raw flesh is no longer observed (Lefevre et al., 2015).

The evolution of the texture parameters throughout shelf life varied, depending on the experimental diets. Whole fish hardness showed no differences on 1 dph, but some differences appeared from 4–10 dph, with the highest for wild fish. At the end of the ice storage period, on the 14 dph, the maximum compression force studied on whole fish was not affected by the dietary treatments (Figure 1). We found no differences from 4–14 dph between each treatment except in diet LL, which was significantly lower on 14 dph, than the other storage days of this diet. A tendency for springiness to diminish can increase during ice storage in all treatments, although differences were only significant between 1–14 dph (data not shown).

The raw fillet hardness was significantly higher in wild fish than fish fed experimental diets during the entire period of ice storage (Figure 2), with differences especially remarkable on 1 dph. All groups showed a significant decrease in values of raw fillet hardness, comparing 1 dph with the other days of sampling. However, while wild fish did not have differences between 4–14 dph, in the other treatments the lowered values obtained were significant, with the highest reduction in fish fed with diets LL+ and LL. Thus, the replacement of fish oil in the diets did not affect instrumental texture parameters when fat content in muscle was not varied, as previously described in gilthead seabream (Matos et al., 2012). On the other hand, the springiness of raw fillet was lower in wild fish but only significantly different from 4–10 dph (data not shown). There were no differences between the other treatments on each dph, or between days of treatment, except 1 and 14 dph in fish fed diet LL+.

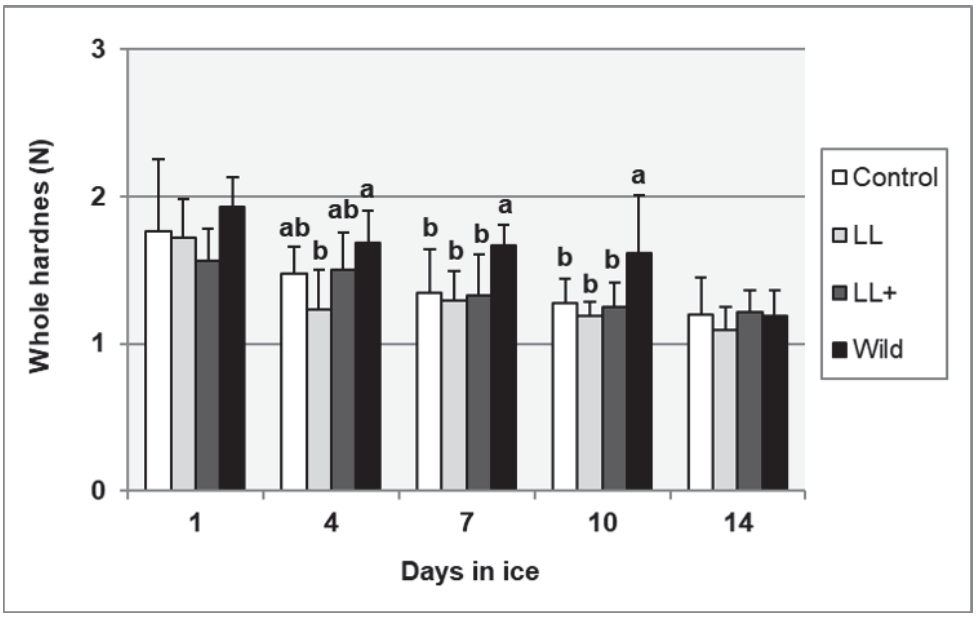

Figure 1. Whole fish hardness (N) of blackspot seabream, wild and fed different diets, throughout ice storage. Different letters in the same day denote statistically significant differences ($P < 0.05$).

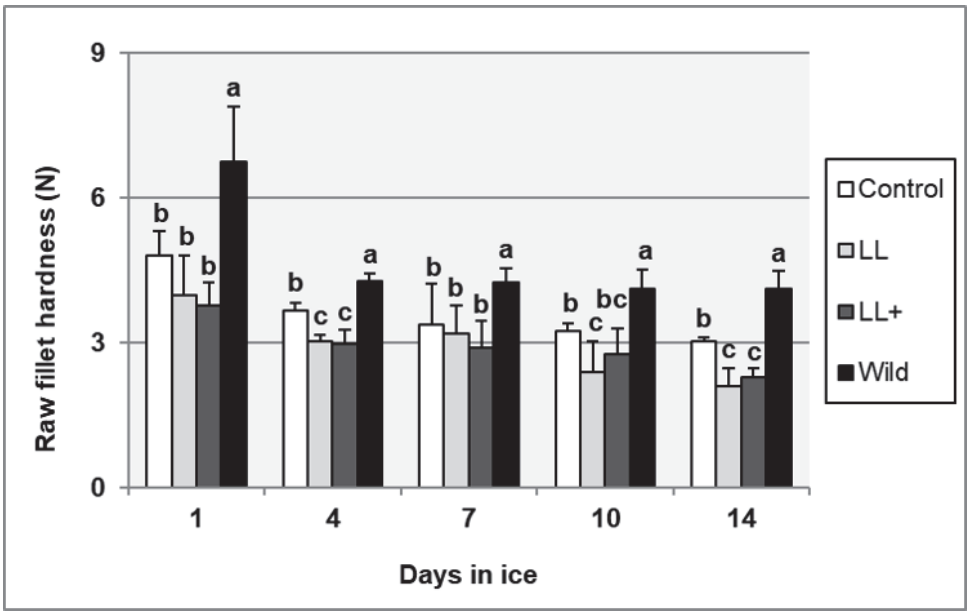

Figure 2. Raw fillet hardness (N) of blackspot seabream, wild and fed different diets, throughout ice storage. Different letters in the same day denote statistically significant differences ($P < 0.05$).

No significant differences in texture parameters were found studying the cooked fillets, neither attributable to the treatment nor the period of ice storage. Only the springiness was statistically lower in wild fish at 1 dph compared with the other treatments (data not

shown). After cooking, the collagen shrinks then softens, whereas the actomyosin complex changes from a soft gel to a firmer denatured complex, making it very difficult to relate the texture attributes of raw flesh to the attributes once the fillet is heated (Hyldig et al., 2001).

The images of white muscle sections from the blackspot seabream fed experimental diets are shown in Figure 3. Morphologically, they are square shaped fibres, with no uniform size, including small diameter fibres intermingled with larger fibres, giving the muscle a characteristic mosaic appearance that results from the hyperplasia process (Johnston, 2001; Castro et al., 2015). Previous studies of blackspot seabream have outlined the muscle growth kinetics, emphasizing hyperplasia as the main relative contributor to the increase of white muscle from larvae to juvenile size (Silva et al., 2008). In wild fish, the density of white muscle fibres was higher than that in the farmed fish, but only significantly different as compared with the muscle of fish fed diets with low lipid content, LL and LL+. Among the farmed fish, those fed the control diet computed the highest number of fibres, significantly superior to those from fish fed diet LL (Figure 4).

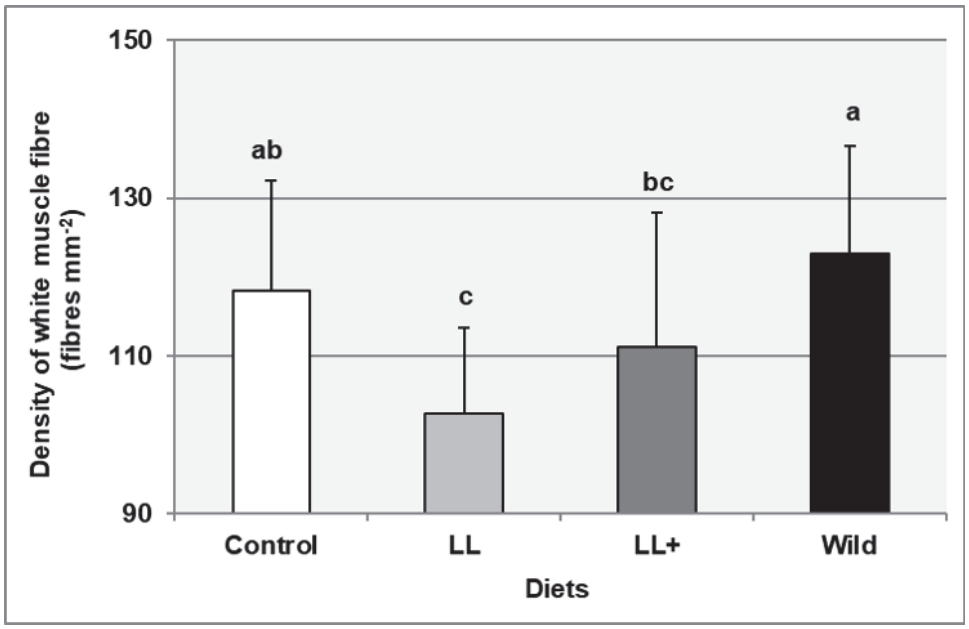

Figure 3. Density of white muscle fibres (fibres mm^{-2}) of blackspot seabream, wild and fed different diets. Different letters in the same day denote statistically significant differences ($P < 0.05$).

Muscle cellularity is considered a determining factor for assessing texture characteristics (Johnston, 1999; Palstra et al., 2011). These studies have pointed out that the density of the muscle fibres holds a positive and significant correlation with texture parameters. High fibre density represents a larger surface-to-volume ratio, and so the connective tissue surrounding each fibre would be relatively more abundant than in a muscle with low fibre density (Periago et al., 2005). In the present study, the wild blackspot seabream, with a large fibre density (Figure 4), showed the highest values of hardness and springiness studied on the raw fillet (Figure 2). This fact does not apply to the cooked fillet, since the correlations with the mechanical resistance parameters are less relevant (Lefevre et al., 2015) as discussed below. Studying wild European sea bass, Periago et al. (2005) found a higher muscle density than in the farmed specimens, and in agreement with the present study, the muscle of wild fish showed the highest values of texture parameters. Similarly, Johnston et al. (2006) reported that wild Atlantic salmon had a firmer texture than that in the farmed fish. However, the authors concluded that with the shear test that was used in their experience, the reported firmness was related to the amount of insoluble hydroxyproline, more than to differences in muscle cellularity. The shear test applies only one deformation to the sample and thus gives no measure of how much of the applied work is absorbed as elastic deformation (Veland et al., 1999), depending mainly on the muscle fibre disposition.

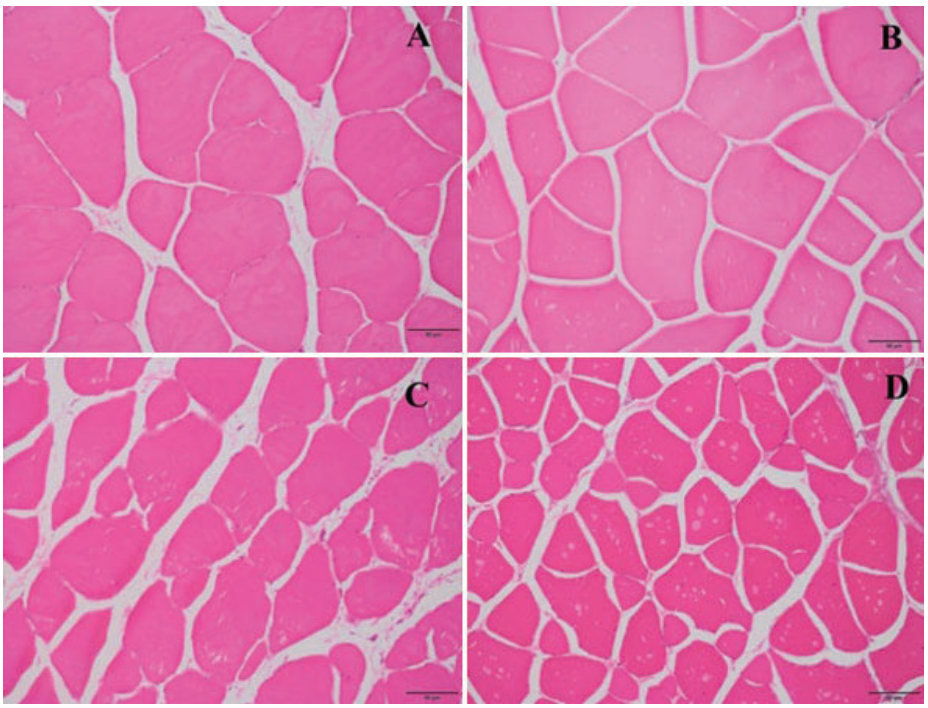

Figure 4. White fibres amount of the blackspot seabream fillet. Haematoxylin–eosin 20×. (A) LL diet. Farmed fish, white muscle. (B) LL+ diet. Farmed fish, white muscle. (C) Control diet. Farmed fish, white muscle. (D) Wild fish, white muscle.

In farmed fish, the number of muscle fibres and fillet texture are influenced by rearing factors, including exercise (Rasmussen et al., 2013), photoperiod (Johnston et al., 2003) and diet formulation (García de la Serrana et al., 2013). Regarding exercise, the improvement of texture parameters as hardness or springiness would be conditioned by the highest white fibre density associated with water velocity into the tank (Li et al., 2016). Light treatment affected muscle growth over the production cycle in salmon, resulting in a large fibre density and a firmer flesh, when continuous photoperiod was applied (Johnston et al., 2003). Finally, and related to diet, Alami-Durante (2010b) reported significant changes in the distribution of the girth of the white muscle fibres, depending on the level of fishmeal substitution by different plant protein sources. That effect could explain those variations in the blackspot seabream fed the experimental diets with low lipid content. Thereby, fish fed diet LL showed a lower fibre density than fish fed diet LL+, due to a lower content of fishmeal. In any case, differences were not significant so that they do not influence the texture profile.

Conversely, the comparison of the results from the fish fed the control diet with the low lipid diets, LL and LL+, showed the highest fibre density on the diet with the highest lipid content, the control diet. Variations of soybean meal content could have promoted these differences and their inclusion would lead to a decrease in the mean and median diameters of muscle fibres, as has been related in species such as rainbow trout (Alami-Durante et al., 2010a). Hence, it seems that the level and origin of the protein of the diet composition influences the muscle growth dynamics, while the replacement of fish oil with vegetable sources has less impact on fibre size (Haugen et al., 2006; Matos et al., 2012).

Therefore, mutually, the size and lipid content of the muscle fibres contributed to the mechanical resistance of the raw fillet; not only the particular responsibility of adipocytes with less resistance to compression, but also the muscle fibres which are bathed in large amounts of lipid and can slide more easily across each other and generate less resistance (Aussanasuwannakul et al., 2011). The collagenous connective tissue structure can contribute to the structural weakening of the muscle (Thakur et al., 2003). Particularly, the highest proportion of intramuscular adipocytes, in the farmed fish muscle, located within the perimysium and myosepta, resulting in a mechanically less resistant tissue as compared with a lean tissue, rich in fibrous proteins (Lefevre et al., 2015). In cooked fillet, the muscle segments tend to slide upon compression because fish has a flaky structure and during heating, the connective tissue that holds the flakes

together dissolves (Castro et al., 2015; Hyldig et al., 2001). This makes the fish muscle fragile with handling after cooking and it separates easily into flakes.

In conclusion, the reduction of fish oil in the commercial diets destined for the sea bream and its replacement by meals, whether of vegetable or fish origin, promotes variable changes in the texture of the fillet during the marketable period. The density of muscle fibres decreases along with the level of dietary fish oil, a fibre reduction boosted with the inclusion of vegetable meal. The reduction of costs of the diet should be weighed, considering the decline in the texture attributes of the fish, detected only in advanced stages of the marketable period.

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CAPÍTULO 5. CONCLUSIONES

1.- Los besugos de pesca presentan claras diferencias sensoriales frente a los de crianza, siendo preponderantes en los primeros las sensaciones de olor y flavor a marisco, mientras que en los segundos dominan las percepciones ligadas al mayor contenido en grasa del filete.

2.- Los porcentajes de EPA en los peces de crianza fueron más altos que en los de pesca, lo que unido al mayor contenido total de grasa en el filete, implica que, por cada porción de 100 g de filete el aporte de EPA+DHA sea de 359 mg, ligeramente superior a los 347 mg del de pesca.

3.- La alimentación de los besugos con dietas bajas en lípidos garantiza el mantenimiento de las propiedades nutritivas beneficiosas para la salud humana en relación a las acumulaciones de EPA+DHA.

4.- A diferencia de lo que sucede con otros ácidos grasos, el DHA reduce paulatinamente su proporción a lo largo de la vida útil.

5.- En una especie magra como es el besugo, la mayor proporción de grasa intramuscular, favorecida por las condiciones de crianza, conlleva una clara variación en la textura del filete, siendo necesario ejercer una menor fuerza de compresión para su deformación.

6.- El área de músculo rojo fue más extensa en los peces de cultivo en las áreas craneal, medial y caudal. En el salvaje, en el musculo rojo se encontraron una mayor cantidad de fibras de calibre más pequeño en las tres áreas estudiadas. Respecto a los cultivados, se evidenció una menor densidad de fibras musculares al disminuir el nivel de inclusión de aceite de pescado en la dieta.