Article

Feed Additives Based on *N. gaditana* and *A. platensis* Blend Improve Quality Parameters of Aquacultured Gilthead Seabream (*Sparus aurata*) Fresh Fillets

María Isabel Sáez [1,2]**, Alba Galafat** [1]**, Silvana Teresa Tapia Paniagua** [3]**, Juan Antonio Martos-Sitcha** [4]**, Francisco Javier Alarcón-López** [1,2] **and Tomás Francisco Martínez Moya** [1,2,*]

[1] Departamento de Biología y Geología, Universidad de Almería, CEIMAR, 04120 Almería, Spain; msc880@ual.es (M.I.S.); agd056@ual.es (A.G.); falarcon@ual.es (F.J.A.-L.)

[2] LifeBioencapsulation S.L., 04131 Almería, Spain

[3] Departamento de Microbiología, CEIMAR, Universidad de Málaga, 29071 Málaga, Spain; stapia@uma.es

[4] Departamento de Biología, Facultad de Ciencias Marinas y Medioambientales, Instituto Universitario de Investigación Marina (INMAR), CEIMAR, Universidad de Cádiz, 11519 Cádiz, Spain; juanantonio.sitcha@uca.es

[*] Correspondence: tomas@ual.es

Abstract: The aim of this research is to explore the potential effects of two microalgae-based additives included in finishing feeds on the quality and shelf-life of seabream fillets. In a 41-day feeding trial, seabream specimens were fed with experimental aquafeeds containing 10% of the bioactive supplements. These additives consisted of a blend of *Nannochloropsis gaditana* and *Arthrospira platensis* biomass, which was utilized as either raw (LB-CB) or enzymatically hydrolyzed (LB-CBplus). A control group received a microalgae-free diet. The results showed that the functional aquafeeds improved the nutritional profile of seabream fillets, increasing protein and PUFA-n3 contents while reducing the atherogenic index, especially for the LB-CBplus treatment. LB-CBplus also enhanced the texture parameters (hardness and chewiness) of fillets during the initial 5 days under cold storage. Regarding skin pigmentation, fillets showed increased greenish and yellowish coloration compared to control fish, mostly attributed to the inclusion of crude algal biomass (LB-CB). Moreover, diets enriched with microalgae additives effectively delayed muscle lipid oxidation processes under refrigeration for up to 12 days, with LB-CBplus exhibiting higher antioxidant effects. These findings highlight the potential of microalgae-based additives to enhance both the nutritional and organoleptic quality of seabream fillets.

Keywords: enzyme hydrolysates; feed additives; fillet quality; finishing diets; microalgal biomass

Key Contribution: Finishing feeds, including microalgae-based additives, were able to improve gilthead seabream fillet skin color and textural parameters compared to commercial diets. Microalgal biomass also improved the fatty acid profile and protected fillet lipids from oxidation during cold storage, especially when pre-treated biomass was considered.

1. Introduction

In recent years, the global demand for seafood has increased considerably. No doubt that the crucial nutritional role that fish play for many communities is responsible for such growth since approximately 3000 million people rely on fish for 20% of their daily protein intake. Some coastal communities are particularly dependent on fish, with reliance levels exceeding 70% [1]. This percentage is expected to rise in the future in a scenario where the figures of extractive fishing have stagnated in the last decade, and consequently, the

aquaculture sector is called upon to meet this demand within the frame of sustainability and climate neutrality without compromising competitiveness.

A wide variety of research strategies are being pursued lately with the aim of enhancing the sustainability of the aquaculture sector, some of them entailing the utilization of species better adapted to climate change or more resistant to mass farming, while others are searching alternative feed sources with low environmental impact or bioactive effects on the animals fed on them. In this context, functional aquafeeds are designed to enhance the adaptive physiological responses of aquatic organisms to both biotic and abiotic factors, as well as improve the quality of the final product reaching the consumer [2,3].

In this regard, an extensive amount of literature has highlighted the potential of microalgae and cyanobacteria as functional additives in feed formulations [4,5]. This is primarily attributed to their richness in bioactive compounds of a diverse nature, including polyphenols, carotenoids, vitamins, and omega-3 polyunsaturated fatty acids (PUFAs), which have been extensively acknowledged for their positive effects on zootechnical and physiological parameters [6–8]. Moreover, studies have demonstrated that the incorporation of algal biomass into finishing feeds can improve fillet quality [9].

However, in order to incorporate algae-based additives into practical commercial products, companies must ensure access to algal raw materials produced on a substantial scale. In this respect, species such as *Nannochloropsis gaditana* and *Arthrospira platensis* are among the most widely produced commercially on a large scale. Furthermore, their inclusion in aquafeeds has confirmed the improvement of numerous objective parameters related to fillet quality [10,11]. However, there are no studies assessing their combined use as a functional additive for seabream.

On the other hand, the constituents present in the cell walls of microalgae may detrimentally impact the utilization of their intracellular components by fish. In this sense, different studies have demonstrated that a simple and cost-effective pre-treatment of algal biomass using cellulolytic enzymes can ameliorate this limitation. Consequently, such enzymatic hydrolysis has been shown to enhance functionality related to digestive processes and antioxidant response [12,13].

The overall hypothesis of this study is to assess the suitability of feeding microalgae-based additives in finishing feeds to improve the quality and shelf-life of sea bream fillets. Specifically, two additives consisting of a combination of the cyanobacteria *A. platensis* and the microalgae *N. gaditana* will be assessed. The basic distinction between them lies in whether an enzymatic pre-treatment of the algal biomass is applied (LB-CBplus) or not (LB-CB).

2. Materials and Methods

2.1. Experimental Diets

Three iso-nitrogenous (42.5% crude protein on dry weight basis, DW) and iso-lipidic (17.3% crude lipid DW) experimental feeds were formulated; two of them contained 100 g kg^{-1} of microalgae-based additives, *LB-ChromaBream* (LB-CB) and LB-*ChromaBream-plus* (LB-CBplus), and a third diet, additive free, was used as control batch (CT). These additives were supplied by LifeBioencapsulation S.L. (Almería, Spain) as freeze-dried concentrated products containing a mixture of two microalgae species (70% *A. platensis* and 30% *N. gaditana*). Algal biomass was used raw (LB-CB) or subjected to enzymatic hydrolysis (LB-CBplus). The enzymatic hydrolysis process was conducted using a combination of commercial proteases under controlled conditions, as outlined in Galafat et al. [12].

The experimental diets were manufactured at CEIA3-Universidad de Almería facilities (Servicio de Piensos Experimentales, https://www.ual.es/universidad/serviciosgenerales/stecnicos/perifericos-convenio/piensos-experimentales (accessed on 25 May 2024); Almeria, Spain) using

standard aquafeed processing procedures. Briefly, feed ingredients and algal biomass were mixed, and then water was added to the mixture (up to 300 g kg^{-1}) to make up the homogeneous dough in a vertical helix ribbon mixer (Sammic BM-10, Sammic, Azpeitia, Spain). The dough was passed through a single screw laboratory extruder (Miltenz 51SP, JSConwell Ltd., Palmerston North, New Zealand) to obtain 5 mm diameter pellets. The extruder barrel consisted of four sections, and the temperature profile in each segment (from inlet to outlet) was 90, 92, 95, and 105 °C, respectively. The feeds were dried (30 °C, 24 h) in a 12 m^3 drying chamber with forced-air circulation (Airfrio, Almería, Spain) and stored at −20 °C until use. The ingredient list and proximal composition of the three experimental diets are provided in Table 1, and their fatty acid profiles are indicated in Table 2.

Table 1. Ingredient composition of the experimental diets.

Ingredient Composition (g 100 g^{-1} Dry Matter)	Diets		
	CT	LB-CB	LB-CBplus
Fish meal LT94 [1]	15.0	15.0	15.0
Lysine [2]	1.2	1.2	1.2
Methionine [2]	0.5	0.5	0.5
Squid meal [3]	1.0	1.0	1.0
Fish meal hydrolysate [4]	0.5	0.5	0.5
LB-ChromaBream [5]	-	10	-
LB-ChromaBream-plus [5]	-	-	10
Wheat gluten [6]	15.0	13.0	13.0
Soybean protein concentrate [7]	35.0	33.0	33.0
Fish oil [8]	5.0	4.5	4.5
Soybean oil [9]	8.0	7.2	7.2
Soybean lecithin [10]	1.0	1.0	1.0
Wheat meal [11]	12.7	8.0	8.0
Choline chloride [12]	0.5	0.5	0.5
Betain [13]	0.5	0.5	0.5
Vitamin and mineral premix [14]	2.1	2.1	2.1
Vitamin C [15]	0.1	0.1	0.1
Guar gum [16]	2.0	2.0	2.0
Crude protein	42.5 ± 0.8	42.1 ± 1.2	43.0 ± 1.7
Crude lipid	14.2 ± 0.3	14.5± 0.2	13.8 ± 0.9
Ash	6.0 ± 0.5	5.8 ± 0.1	5.9 ± 0.0

Dietary treatments: CT: control diet; LB-CB: diet including 100 g kg^{-1} *LB-Chromabream* additive; LB-CBplus: diet including 100 g kg^{-1} *LB-ChromabreamPlus* additive. [1] (protein: BA.4%; lipid: 12.G%), Norsildemel (Bergen, Norway); [2] Suysegala (Sevilla, Spain); [3] Bacarel (UL); [4] CPSP (Sopropeche, France); [5] Lifebioencapsulation SL (Almería, Spain); [6] (protein: 7B.0%; lipid: 1.A%), Lorca Nutricion Animal (Murcia, Spain); [7] (protein: 50.0%; lipid: 1.0%) Lorca Nutricion Animal (Murcia, Spain); [8] AFAMPES 117ECA (AFAMSA, Pontevedra, Spain); [9] Aceites el Niño (Málaga, Spain); [10] Lecico P700 (Lecico GmbC, EE); [11] (protein: 12.0%; lipid: 2.0%), local provider; [12,13] Sigma-Aldrich (Madrid, Spain); [14,15] vitamin and mineral premix: vitamins (IU or mg kg^{-1} premix): vitamin A (retinyl acetate), 2000,000 IU; vitamin EG (EL cholecalciferol), 200,000 IU; vitamin E, 10,000 mg; vitamin LG (menadione sodium bisulphite), 2500 mg; vitamin B1 (thiamine hydrochloride), G000 mg; vitamin B2 (riboKavin), G000 mg; calcium pantothenate, 10,000 mg; nicotinic acid, 20,000 mg; vitamin BB (pyridoxine hydrochloride), 2000 mg; vitamin BA (folic acid), 1500 mg; vitamin B12 (cyanocobalamin), 10 mg vitamin C (biotin), G00 mg; inositol, 50,000 mg; betaine, 50,000 mg; vitamin C (ascorbic acid), 50,000 mg. Minerals (mg kg^{-1} premix): Co (cobalt carbonate), B5 mg; Cu (cupric sulfate), A00 mg; Fe (iron sulfate), B00 mg; I (potassium iodide), 50 mg; Mn (manganese oxide), AB0 mg; Se (sodium selenite), 1 mg; Nn (zinc sulfate) 750 mg; Ca (calcium carbonate), 18B,000 mg; LCl,

24,100 mg; NaCl 40,000 mg; excipient sepiolite, colloidal silica (LifeBioencapsulation premix); [16] EPSA (Sevilla, Spain).

Table 2. Fatty acid profile of the experimental diets (% of total fatty acids).

	Experimental Diets			
	CT	**LB-CB**	**LB-CBplus**	***p***
14:0	1.43 ± 0.01 [a]	1.62 ± 0.01 [b]	1.58 ± 0.02 [b]	0.001
16:0	14.09 ± 0.05 [a]	14.79 ± 0.06 [b]	14.66 ± 0.08 [b]	0.003
18:00	4.44 ± 0.04 [b]	4.3 ± 0.01 [a]	4.35 ± 0.02 [ab]	0.027
16:1n-7	2.14 ± 0.04	1.76 ± 0.67	2.26 ± 0.02	n.s.
18:1n-7	1.23 ± 0.04	1.25 ± 0.01	1.26 ± 0.01	n.s.
18:1n-9	21.03 ± 0.06 [b]	20.42 ± 0.09 [a]	20.5 ± 0.06 [a]	0.006
20:1n-9	0.05 ± 0.07	0.05 ± 0.07	0.05 ± 0.07	n.s.
18:2n-6	37.86 ± 0.12 [b]	36.53 ± 0.09 [a]	36.57 ± 0.26 [a]	0.008
18:3n-3	0.96 ± 0.14 [a]	1.32 ± 0.01 [b]	1.24 ± 0.00 [ab]	0.043
16:2n4	0.29 ± 0.06	0.3 ± 0.06	0.29 ± 0.06	n.s.
16:3n4	0.44 ± 0.06	0.44 ± 0.06	0.44 ± 0.06	n.s.
18:4n-3	0.86 ± 0.02	0.82 ± 0.01	0.85 ± 0.01	n.s.
20:4n-6	0.73 ± 0.22	0.56 ± 0.01	0.57 ± 0.02	n.s.
20:4n-3	0.15 ± 0.01	0.14 ± 0.02	0.14 ± 0.01	n.s.
20:5n-3 (EPA [1])	2.99 ± 0.01	2.98 ± 0.01	2.85 ± 0.20	n.s.
22:5n-3	0.38 ± 0.01	0.38 ± 0.01	0.38 ± 0.01	n.s.
22:6n-3 (DHA [2])	7.49 ± 0.12	7.37 ± 0.03	7.44 ± 0.07	n.s.
ΣSFAs [3]	19.96 ± 0.02 [a]	20.71 ± 0.07 [b]	20.59 ± 0.07 [b]	0.002
ΣMUFAs [4]	24.45 ± 0.01	23.48 ± 0.64	24.07 ± 0.02	n.s.
ΣPUFAs [5]	56.3 ± 0.09 [b]	54.84 ± 0.09 [a]	54.84 ± 0.53 [a]	0.029
ΣPUFAs n-3	12.83 ± 0.27	13.02 ± 0.06	12.91 ± 0.27	n.s.
ΣPUFAs n-6	38.6 ± 0.34 [b]	37.08 ± 0.1 [a]	37.15 ± 0.29 [a]	n.s.
n-3/n-6	0.33 ± 0.01	0.35 ± 0.00	0.35 ± 0.00	n.s.
EPA/DHA	0.40 ± 0.01	0.40 ± 0.00	0.38 ± 0.02	n.s.
PUFA/SFA	2.82 ± 0.01 [b]	2.64 ± 0.01 [a]	2.66 ± 0.01 [a]	0.006

Dietary treatments: CT: control diet; LB-CB: diet including 100 g kg⁻¹ *LB-Chromabream* additive; LB-CBplus: diet including 100 g kg⁻¹ *LB-ChromabreamPlus* additive. Values with different lowercase superscripts indicate significant differences attributed to dietary treatments ($p < 0.05$). [1] EPA: eicosapentaenoic acid; [2] DHA: docosahexaenoic acid. [3] SFAs: saturated fatty acids; [4] MUFAs: monounsaturated fatty acids; [5] PUFAs: polyunsaturated fatty acids. Values are expressed as average ±SD (n = 3). n.s.: not significant.

2.2. Fish Maintenance and Experimental Design

Gilthead seabream (*Sparus aurata*) juveniles were provided by a commercial firm (CUPIBAR, Chiclana de la Frontera, Cádiz). The feeding trial was carried out at *Servicios Centrales de Investigacion en Cultivos Marinos* (SCI-CM, CASEM, Universidad de Cadiz, Spain). All experimental procedures complied with the Guidelines of the European Union (Directive 2010/63/EU) and the Spanish regulations (Real Decreto 53/2013, as amended by RD 118/2021) on the protection of laboratory animals. A total of 108 seabream juveniles (182.5 ± 1.8 g initial body weight) were randomly distributed in 400 L tanks (triplicate tanks per dietary treatment) (3 dietary treatments × 3 tanks per treatment × 12 fish per tank).

All fish were fed with a CT diet during a 15-d acclimation period prior to the beginning of the feeding trial. Afterwards, the different experimental diets were offered ad libitum once per day during a 41-d period. The feeding trial was carried out in an open flow circuit, keeping seawater (36‰ salinity) renewal rate at 500 L h⁻¹ and ammonia and

nitrite values (<0.1 mg L^{-1}) suitable for gilthead seabream culture. Animals were kept under a natural photoperiod at our latitude (36°31′45″ N, 6°11′31″ W, from January to May 2020), and the water temperature was kept at 19.0 ± 1.0 °C.

At the end of the feeding trial (41 days), 8 fish per tank (24 specimens per dietary treatment) were withdrawn, individually weighed and measured, and then killed by anesthetic overdose (1 mL L^{-1} 2-phenoxyethanol, Sigma-Aldrich) followed by spine severing. The rest of the animals were kept for a different study [14]. Immediately after slaughtering, specimens were gutted, filleted, and then packed in transparent sterile polyethylene bags. The bags were stored in a cold room (4 °C ± 1 °C) for a period of 12 d with the aim of assessing changes in quality parameters under refrigeration. Samples were withdrawn from each lot at 1, 2, 5, 7, 9, and 12 days *post-mortem* (*dpm*). At each sampling time, 4 fillets per treatment were withdrawn to determine skin and flesh instrumental color, texture profile analysis (TPA), pH, water holding capacity (WHC), and lipid oxidation. At 1 *dpm*, subsamples were obtained for proximal composition and fatty acid profile analysis.

2.3. Fillet Proximate Composition and Fatty Acid Analysis

Proximate analysis (dry matter, ash, and crude protein, N × 6.25) of feeds and muscle samples were determined according to AOAC [15] procedures. Lipids were extracted following the methodology proposed by Folch [16] using chloroform/methanol (2:1 *v/v*) as a solvent, and total lipid content was calculated gravimetrically. The fatty acid (FA) profile was determined by gas chromatography according to Rodríguez-Ruiz et al. [17] by means of a gas chromatograph (Hewlett Packard, 4890 Series II, Hewlett Packard Company, Avondale, PA, USA) using a modification of the direct transesterification method described by Lepage and Roy [18] that requires no prior separation of the lipid fraction.

From the FA profile of fish muscle, the index of atherogenicity (IA) and the index of thrombogenicity (IT) were calculated as described by Senso et al. [19] as follows: index of atherogenicity (IA) = (12:0 + 4 × 14:0 + 16:0)/[(n6 + n3) PUFAs + 18:1 + other MUFAs] and index of thrombogenicity (IT) = (14:0 + 16:0 + 18:0)/[(0.5 × 18:1) + (0.5 × ΣMUFAs) + (0.5 × n6-PUFAs) + (3 × n3-PUFAs) + (n3/n6)], where MUFAs and PUFAs stand for monounsaturated fatty acids and polyunsaturated fatty acids, respectively.

2.4. Post-Mortem Changes during Cold Storage

2.4.1. Lipid Oxidation

Lipid oxidation was estimated by thiobarbituric acid-reactive substances (TBARS) analysis throughout the 12 d storage period. TBARS were measured in muscle samples according to the spectrophotometric method by Buege and Aust [20]. Muscle samples (1 g) were homogenized in 4 mL 50 mM NaH$_2$PO4, 0.1% (*v/v*) Triton X-100 solution. The resulted mixture was centrifuged (10,000× *g*, 20 min, 4 °C), and supernatants were mixed in a ratio of 1:5 (*v/v*) with a 2-thiobarbituric acid (TBA) reagent (0.375% *w/v* TBA, 15% *w/v* TCA, 0.01% *w/v* 2,6-dibutyl hydroxytoluene (BHT) and 0.25 N HCl) and heated (100 °C) for 15 min. Subsequently, the mixtures were centrifuged (3600× *g*, 10 min, 4 °C, Sigma 2-16PK, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany), and the absorbance of supernatants was measured spectrophotometrically at 535 nm (Multiskan Sky, Thermo Scientific, Waltham, MA, USA). The amount of TBARS was expressed as an mg of malonyl dialdehyde (MDA) per kg of fresh muscle after comparing it with an MDA standard.

2.4.2. Instrumental Color Determination

Pigmentation was measured thrice on the skin and flesh sides (dorsal portion) of fillets by the L*, a*, and b* system [21] using a Minolta Chroma meter CR400 device (Minolta, Osaka, Japan). The color parameters of lightness (L*, on a 0-100 point scale from black to white), redness-greenness (a*, estimates the position between red, positive values,

and green, negative values), and yellowness-blueness (b*, estimates the position between yellow, positive values, and blue, negative values) were recorded.

2.4.3. Texture Profile Analysis (TPA)

Fillet texture was measured by compression of the anterior area to the dorsal fin above the lateral line of fillets using a Texture Analyser (TXT2 plus "Stable Micro System") equipped with a load cell of 5 kN and controlled with Texture Expert Exceed 2.52 software (Stable Micro Systems, Surrey, UK). Muscle samples (thickness from 12 to 15 mm) were subjected to two consecutive cycles of 25% compression with 5 s between cycles, in which a 20 mm cylindrical probe was used for pressing downwards into the fillet at a constant speed of 1 mm/s. The textural parameters of hardness, springiness, cohesiveness, gumminess, chewiness, and resilience were calculated according to Bourne [22].

2.4.4. pH and Water Holding Capacity (WHC)

Muscle pH was determined in the anterior part of the dorsal muscle by means of a penetration electrode (Crison, model GLP 21; sensitivity 0.01 pH units) as described in Suárez et al. [23]. WHC (expressed in percentage) was calculated from a piece (1 cm^3) of dorsal muscle as the difference between the initial percentage of water and the percentage of water released after centrifugation (630× g, 30 min, 10 °C, Sigma 2-16PK, Osterode am Harz, Germany) according to Suárez et al. [23].

2.5. Statistics

The effect of the categorical variables "additive" and "storage time", as well as their interactions, were determined for each numeric parameter studied by fitting a generalized linear statistical model (GLM analysis) that relates measured parameters to predictive factors using specific software (SPSS 22, IBM Corporation Inc., Armonk, NY, USA). Least square means were tested for differences using Fisher's least significant difference (LSD) procedure. Unless otherwise specified, a significance level of 95% was considered to indicate statistical difference ($p < 0.05$). When measurements were expressed as a percentage (e.g., fatty acids and WHC), arcsine transformation of their square root was carried out to normalize data prior to the statistical analysis.

3. Results

3.1. Growth Performance and Body Composition

Fish mortality was below 5% in all tanks at the end of the feeding trial. After 41 d, LB-CBplus fish showed significantly higher final body weight and specific growth rate (SGR) compared to the control (CT) batch (Table 3, data reported in [14]). On the contrary, the fillet yield recorded was similar for all the experimental diets.

Table 3. Growth performance and somatic indexes at day 41 of the feeding trial.

Parameters	CT	LB-CB	LB-CBplus	*p*
Initial weight (g)	182.50 ± 0.16	182.60 ± 0.30	182.60 ± 0.17	n.s.
Final weight (g)	233.0 ± 0.66 [a]	240.8 ± 3.20 [ab]	247.7 ± 2.42 [b]	0.013
SGR * (%)	0.59 ± 0.01 [a]	0.67 ± 0.03 [ab]	0.74 ± 0.02 [b]	0.008
Fillet yield (%)	60.41 ± 2.43	59.96 ± 1.64	59.12 ± 2.50	n.s.

Dietary treatments: CT: control diet; LB-CB: diet including 100 g kg^{-1} *LB-Chromabream* additive; LB-CBplus: diet including 100 g kg^{-1} *LB-ChromabreamPlus* additive. * SGR: specific growth rate. Values with different lowercase superscript indicate significant differences attributed to dietary treatments ($p < 0.05$). n.s.: not significant.

Regarding muscle proximal composition (Table 4), differences in protein and lipid contents were observed among experimental groups. In this sense, dietary treatments, including microalgae-based additives (LB-CB and LB-CBplus), yielded a lower muscle

total lipid content compared to the control fillets ($p < 0.01$). On the other hand, protein content was higher in the LB-CBplus lot ($p = 0.012$) compared to the other two treatments.

Table 4. Effects of the dietary inclusion of LB-CB and LB-CBplus additives on muscle composition of seabream fillets at 41 d of the feeding trial (%).

	CT	LB-CB	LB-CBplus	*p*
Crude protein	19.82 ± 0.26 [a]	20.13 ± 0.12 [a]	20.95 ± 0.20 [b]	0.012
Crude lipid	3.26 ± 0.03 [b]	3.01 ± 0.06 [a]	2.88 ± 0.03 [a]	<0.001
Ash	6.51 ± 0.02	6.53 ± 0.07	6.51 ± 0.13	n.s.
Moisture	71.91 ± 0.56	71.60 ± 0.72	70.86 ± 0.52	n.s.

Dietary treatments: **CT:** control diet; LB-CB: diet including 100 g kg^{-1} *LB-Chromabream* additive; LB-CBplus: diet including 100 g kg^{-1} *LB-ChromabreamPlus* additive. Values with different lowercase superscript indicate significant differences attributed to dietary treatments ($p < 0.05$). n.s.: not significant.

3.2. Muscle Fatty Acid Profile

The muscle fatty acid (FA) composition of gilthead seabream fillets is summarized in Table 5. Polyunsaturated fatty acids (PUFAs) were predominant in seabream fillets at the end of the feeding trial, regardless of the dietary treatment considered (39.5–41.5% total FAs), followed by monounsaturated fatty acids (MUFAs, 33.9–35.7%) and then saturated fatty acids (SFAs, 22–23%).

Table 5. Effects of the dietary inclusion of LB-CB and LB-CBplus additives on the fatty acid (FA) profile of gilthead seabream (*S. aurata*) muscles after a 41-d feeding trial (% of total FAs).

Fatty Acids (%)	CT	LB-CB	LB-CBplus	*p*
14:0	2.84 ± 0.02 [c]	2.77 ± 0.01 [b]	2.64 ± 0.01 [a]	<0.001
16:0	15.58 ± 0.06 [a]	15.85 ± 0.11 [b]	15.90 ± 0.02 [b]	0.043
18:00	3.91 ± 0.01 [ab]	3.88 ± 0.04 [a]	3.99 ± 0.00 [b]	0.049
16:1n-7	5.30 ± 0.05	5.01 ± 0.24	4.95 ± 0.01	n.s.
18:1n-7	2.92 ± 0.00 [b]	2.89 ± 0.02 [b]	2.78 ± 0.00 [a]	0.003
18:1n-9	25.36 ± 0.12 [b]	25.32 ± 0.09 [b]	24.44 ± 0.06 [a]	0.003
20:1n-9	2.16 ± 0.01	1.20 ± 0.84	1.77 ± 0.10	n.s.
18:2n-6	14.64 ± 0.07 [a]	14.98 ± 0.05 [b]	15.94 ± 0.00 [c]	<0.001
18:3n-3	2.14 ± 0.05 [ab]	2.20 ± 0.04 [b]	2.04 ± 0.01 [a]	0.043
16:2n4	0.42 ± 0.22	0.55 ± 0.01	0.53 ± 0.02	n.s.
16:3n4	0.53 ± 0.01	0.54 ± 0.00	0.52 ± 0.00	n.s.
18:4n-3	0.73 ± 0.01	1.30 ± 0.70	0.72 ± 0.00	n.s.
20:4n-6	0.96 ± 0.01	0.92 ± 0.07	0.94 ± 0.02	n.s.
20:4n-3	4.80 ± 0.01 [a]	5.01 ± 0.02 [b]	5.04 ± 0.03 [b]	0.003
20:5n-3 (EPA [1])	1.25 ± 0.08 [a]	1.63 ± 0.07 [b]	1.76 ± 0.14 [b]	0.027
22:5n-3	2.48 ± 0.01 [c]	2.34 ± 0.02 [b]	2.26 ± 0.00 [a]	<0.001
22:6n-3 (DHA [2])	10.24 ± 0.02 [a]	10.17 ± 0.11 [a]	10.99 ± 0.03 [b]	0.002
ΣSFAs [3]	22.34 ± 0.09	22.51 ± 0.15	22.53 ± 0.03	n.s.
ΣMUFAs [4]	35.73 ± 0.18	34.42 ± 0.97	33.94 ± 0.03	n.s.
ΣPUFAs [5]	39.4 ± 0.15 [a]	39.74 ± 0.03 [b]	41.46 ± 0.04 [c]	<0.001
ΣPUFAs n3	21.65 ± 0.07 [a]	22.64 ± 0.09 [b]	22.82 ± 0.12 [b]	<0.001
ΣPUFAs n6	15.6 ± 0.08 [a]	15.90 ± 0.02 [b]	16.88 ± 0.02 [c]	<0.001

n3/n6	1.39 ± 0	1.42 ± 0.06	1.35 ± 0.01	n.s.
EPA/DHA	0.12 ± 0.01 [a]	0.16 ± 0.01 [b]	0.16 ± 0.01 [b]	0.035
AI [6]	0.38 ± 0.01 [b]	0.38 ± 0.01 [b]	0.36 ± 0.01 [a]	0.027
TI [7]	0.24 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	n.s.

Dietary treatments: CT: control diet; LB-CB: diet including 100 g kg^{-1} *LB-Chromabream* additive; LB-CBplus: diet including 100 g kg^{-1} *LB-ChromabreamPlus* additive. Values with different lowercase superscripts indicate significant differences attributed to dietary treatments ($p < 0.05$). [1] EPA: eicosapentaenoic acid; [2] DHA: docosahexaenoic acid. [3] SFAs: saturated fatty acids; [4] MUFAs: monounsaturated fatty acids; [5] PUFAs: polyunsaturated fatty acids; [6] AIs: atherogenic indices and [7] TIs: thrombogenic indices, as explained in the M&M section. Values are expressed as average ±SD (n = 9 fish per dietary treatment). Values with different lowercase superscript indicate significant differences attributed to dietary treatments ($p < 0.05$). n.s.: not significant.

The main effect of the supplementation with the functional additives on muscle lipids can be summarized as LB-CBplus fillets causing an increase in polyunsaturated (ΣPUFA) content, both in n3-PUFA and n6-PUFA, while the lowest values for both categories were found in CT batch. The rest of the fatty acid groups were not affected by additive supplementation at the end of the feeding trial. Regarding n3-PUFA contents, the EPA and EPA/DHA ratios were significantly higher in specimens fed with additive-enriched diets ($p = 0.027$ and $p < 0.001$ for LB-CB and LB-CBplus, respectively). Meanwhile, LB-CBplus fillets yielded higher DHA content and the lowest value for the AI ($p = 0.002$ and 0.027, respectively).

3.3. TBARS Content

Post-mortem changes in TBARS contents observed in seabream fillets during cold storage at 4 °C are shown in Figure 1. In general, muscle lipid oxidation showed differences attributable to the "additive" and "storage time" factors, as well as their interactions.
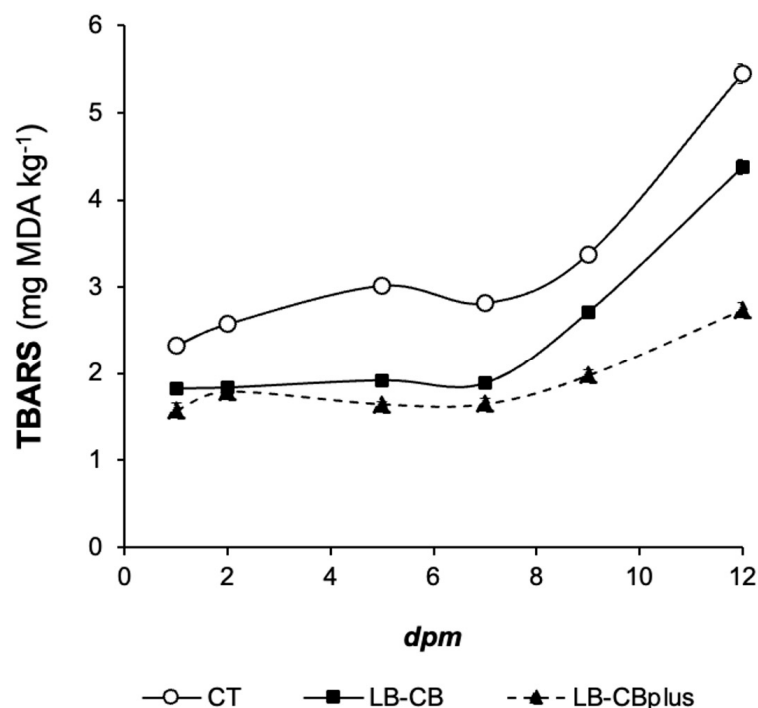

Figure 1. Time course of muscle lipid oxidation (estimated as TBARS content) of seabream fillets throughout a 12-d cold (4 °C) storage period. CT: control diet; LB-CB: diet including 100 g kg^{-1} LB-Chromabream additive; LB-CBplus: diet including 100 g kg^{-1} LB-ChromabreamPlus additive. Values are expressed as average ±SD (n = 4).

TBARS content increased markedly over time in all the experimental lots ($p < 0.01$). During the complete storage period, the CT batch showed significantly higher values than the additive-supplemented batches. In addition, differences were also observed between both additive-supplemented diets given that LB-CBplus yielded significantly higher antioxidant response than LB-CB from day 5 onwards ($p < 0.01$).

3.4. Instrumental Color Assessment

The influence of the dietary treatments on the changes in fillet skin color parameters throughout the storage time is summarized in Figure 2. Significant differences for parameters a* and b* were observed, showing that fish fed with algae-based additives have higher greenness (lower a*) and yellowness (higher b*) skin compared to the CT batch. For parameter b*, these differences were more evident in the LB-CB group (crude microalgae-based additive) than in LB-CB-plus (hydrolyzed microalgae-based additive) up to 7 *dpm* (Figure 2C). Meanwhile, cold storage decreased skin brightness (L*) in all batches, without any difference among the experimental treatments.
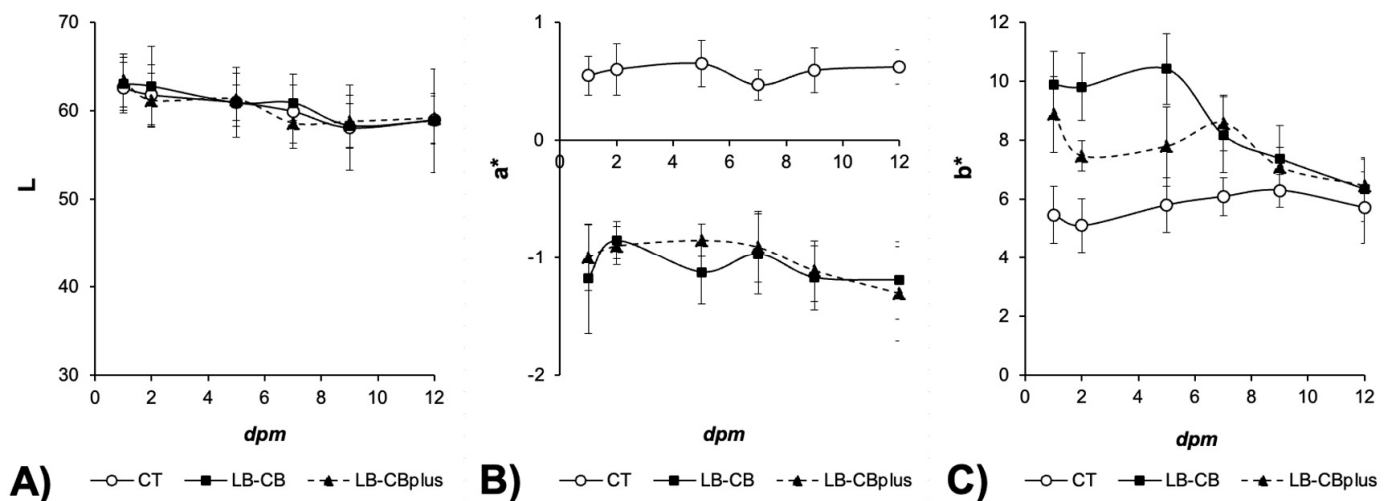

Figure 2. Changes in L* (**A**), a* (**B**), and b* (**C**) skin color parameters of seabream fillets throughout a 12 d cold (4 °C) storage period. CT: control diet; LB-CB: diet including 100 g kg^{-1} LB-Chromabream additive; LB-CBplus: diet including 100 g kg^{-1} LB-ChromabreamPlus additive. Values are expressed as average ±SD (n = 4).

Regarding fillet flesh color (Figure 3), the experimental variables (diet and storage time) also caused a certain impact on the parameters measured, although less markedly than described for the skin side. Thus, flesh brightness (L*) oscillated during cold conservation, and no clear trend could be observed. Parameter a* was not influenced by the dietary treatments, although values decreased from the beginning to the end of the storage period in all the experimental lots. On the other hand, some differences attributable to dietary treatments were observed for parameter b*, and, therefore, values for CT fillets increased markedly with storage time (increased yellowish); both additive-enriched (LB-CB and LB-CB-plus) lots were able to prevent such increase throughout the complete storage time (Figure 3B).
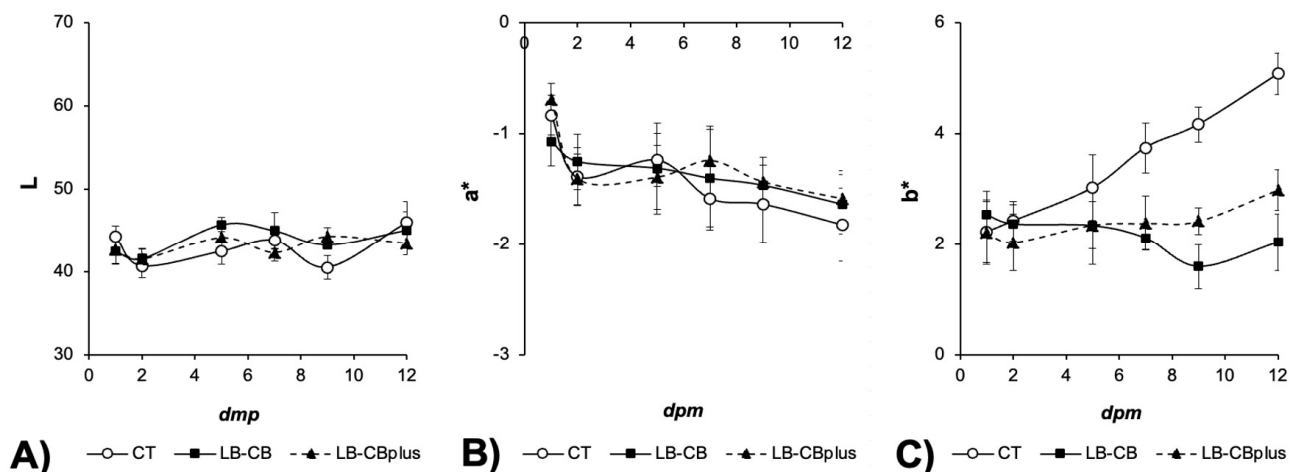
Figure 3. Changes in L* (**A**), a* (**B**), and b* (**C**) flesh color parameters of seabream fillets throughout a 12 d cold (4 °C) storage period. CT: control diet; LB-CB: diet including 100 g kg^{-1} LB-Chromabream additive; LB-CBplus: diet including 100 g kg^{-1} LB-ChromabreamPlus additive. Values are expressed as average ±SD (n = 4).

3.5. TPA Determinations

The effects of additive supplementation on fillet textural parameters are outlined in Figure 4 for the hardness parameter and Table 6 for the rest of the textural attributes. Overall, a noticeable influence of the functional diets on the parameters of hardness, gumminess, and chewiness could be observed during the initial days of fillet cold storage (up to 4 *dpm*). Nevertheless, from the fifth day onwards, the values were similar among treatments. In this context, at the initial stage (1 and 2 *dpm*), seabream fillets fed with feeds supplemented with microalgae-based additives exhibited higher hardness compared to CT fillets. This effect was more evident in the LB-CBplus group ($p < 0.01$). A similar trend was observed for gumminess and chewiness parameters. The rest of the textural attributes were not affected by the dietary treatments.
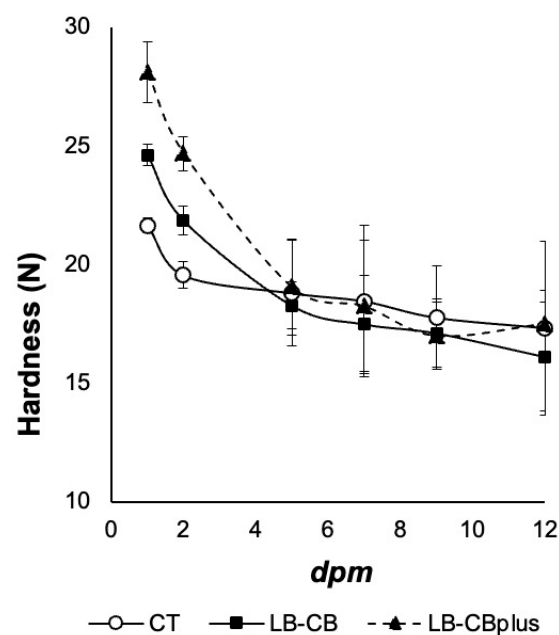

Figure 4. Time course of fillets hardness throughout a 12 d cold (4 °C) storage period. CT: control diet; LB-CB: diet including 100 g kg^{-1} LB-Chromabream additive; LB-CBplus: diet including 100 g kg^{-1} LB-ChromabreamPlus additive. Values are expressed as average ±SD (n = 4).

With regard to the influence of storage time, roughly, hardness, cohesiveness, gumminess, and chewiness attributes decreased during the *post-mortem* cold preservation in all the experimental lots.

Table 6. Time-course of texture profile analysis (TPA) parameters in seabream fillets during a 12-d cold storage (4 °C) period.

	dpm	CT	LB-CB	LB-CBplus	*p*
	1	0.78 ± 0.07	0.79 ± 0.04	0.84 ± 0.04 [2]	n.s.
	2	0.81 ± 0.04	0.77 ± 0.04	0.84 ± 0.05 [12]	n.s.
	5	0.75 ± 0.04	0.77 ± 0.04	0.78 ± 0.01 [1]	n.s.
Springiness (mm)	7	0.75 ± 0.03	0.76 ± 0.05	0.77 ± 0.04 [12]	n.s.
	9	0.72 ± 0.04 [a]	0.73 ± 0.05 [ab]	0.81 ± 0.02 [b,12]	0.045
	12	0.77 ± 0.03	0.77 ± 0.02	0.76 ± 0.04 [12]	n.s.
	P	n.s.	n.s.	0.017	
	1	0.42 ± 0.02 [2]	0.41 ± 0.03 [2]	0.45 ± 0.02 [3]	n.s.
	2	0.42 ± 0.02 [2]	0.40 ± 0.02 [2]	0.42 ± 0.02 [23]	n.s.
	5	0.39 ± 0.04 [12]	0.39 ± 0.00 [12]	0.39 ± 0.03 [12]	n.s.
Cohesiveness	7	0.38 ± 0.01 [12]	0.37 ± 0.02 [1]	0.37 ± 0.02 [1]	n.s.
	9	0.38 ± 0.01 [12]	0.37 ± 0.03 [1]	0.37 ± 0.06 [1]	n.s.
	12	0.36 ± 0.01 [1]	0.37 ± 0.02 [1]	0.37 ± 0.02 [1]	n.s.
	P	0.015	0.024	0.009	
	1	9.08 ± 0.44 [a,3]	10.19 ± 0.74 [b,2]	12.63 ± 0.86 [b,3]	<0.001
	2	8.20 ± 0.41 [a,23]	8.83 ± 0.38 [ab,12]	10.41 ± 0.83 [b,23]	0.001
	5	7.40 ±1.13 [12]	7.04 ± 0.41 [12]	7.42 ±0.82 [12]	n.s.
Gumminess (N/mm2)	7	7.11 ± 1.34 [1]	6.49 ± 0.99 [12]	6.80 ± 0.90 [1]	n.s.
	9	6.79 ± 0.67 [1]	6.28 ± 0.95 [12]	6.30 ± 0.95 [1]	n.s.
	12	6.34 ± 1.48 [1]	5.95 ± 0.78 [1]	6.43 ± 0.54 [1]	n.s.
	P	0.003	0.014	<0.001	
	1	7.30 ± 0.35 [a,2]	8.30 ± 0.39 [b,2]	11.13 ± 0.95 [c,3]	<0.001
	2	6.67 ± 0.59 [a,2]	7.12 ± 0.46 [a,12]	8.85 ± 0.50 [b,2]	0.001
	5	5.51 ± 0.78 [1]	5.44 ± 0.40 [1]	5.76 ± 0.60 [1]	n.s.
Chewiness (N.mm)	7	5.30 ± 1.00 [1]	4.93 ± 0.81 [1]	5.23 ± 0.61 [1]	n.s.
	9	4.89 ± 0.62 [1]	4.63 ± 0.92 [1]	5.28 ± 0.99 [1]	n.s.
	12	4.88 ± 1.21 [1]	4.88 ± 0.36 [1]	4.89 ± 0.55 [1]	n.s.
	P	0.001	0.036	<0.001	
	1	0.17 ± 0.02	0.17 ± 0.02	0.19 ± 0.03	n.s.
	2	0.17 ± 0.04	0.16 ± 0.02	0.19 ± 0.04	n.s.
	5	0.17 ± 0.02	0.16 ± 0.01	0.15 ± 0.02	n.s.
Resilience (N/mm)	7	0.17 ± 0.05	0.15 ± 0.01	0.15 ± 0.01	n.s.
	9	0.15 ± 0.02	0.15 ± 0.02	0.14 ± 0.02	n.s.
	12	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	n.s.
	P	n.s.	n.s.	n.s.	

Dietary treatments: CT: control diet; LB-CB: diet including 100 g kg^{-1} LB-Chromabream additive; LB-CBplus: diet including 100 g kg^{-1} LB-ChromabreamPlus additive. Superscript numbers indicate differences attributable to storage time within each treatment. Superscript lower-case letters indicate differences attributable to treatments within each storage time (*p* < 0.05). Values are expressed as average ±SD (n = 4 fillets per dietary treatment and sampling time). dpm: days post-mortem. n.s.: not significant.

3.6. pH and WHC

Post-mortem changes in pH values observed in seabream fillets during cold storage at 4 °C are shown in Figure 5A. The inclusion of either of the additives in aquafeeds was responsible for differences in this parameter, and thus, CT fillets yielded higher muscle pH values, which were only statistically significant at 5 and 9 *dpm* (p = 0.002 and 0.021, respectively). Storage time increased pH values in all batches at the end of the assay (p < 0.01).
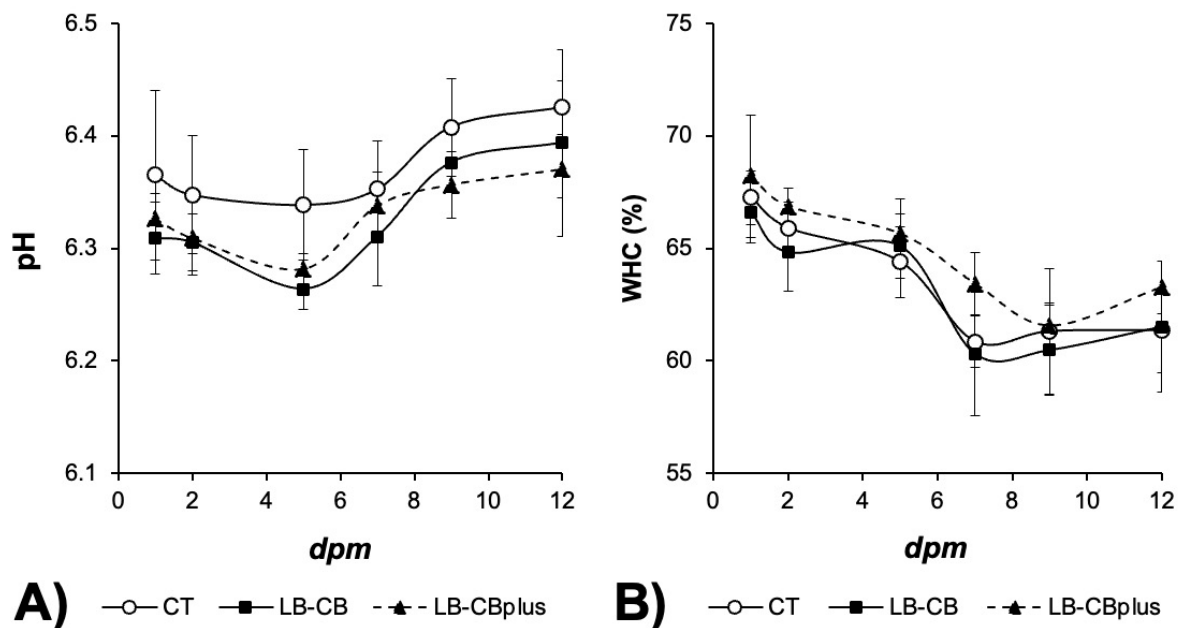

Figure 5. Changes in pH (**A**) and WHC (**B**) of seabream fillets throughout a 12-d cold (4 °C) storage period. CT: control diet; LB-CB: diet including 100 g kg^{-1} LB-Chromabream additive; LB-CBplus: diet including 100 g kg^{-1} LB-ChromabreamPlus additive. Values are expressed as average ±SD (n = 4).

On the other hand, fillet WHC decreased during the 12-d period in all lots (Figure 5B), and no changes attributable to diet were recorded.

4. Discussion

In recent years, the literature has reported that many of the bioactive substances present in microalgae biomass can cause a wide range of beneficial biological activities on farmed fish species, even when included at low levels in aquafeeds. In other words, the interest in such supplementation is predominantly based on its functional effects, not so much on its importance as a macroingredient in feeds. Among the many beneficial effects described for microalgae biomass in aquaculture, increasing interest is being given to its influence on objective quality parameters of fish fillets as a product for human consumption [24–28]. In this regard, a practical approach available in fish farming is the use of fattening diets, supplied for a short period close to the end of the production cycle, which allows a modification of fillet characteristics, both from the point of view of nutritional and organoleptic quality. This strategy is based on the fact that variations in the characteristics of the diet can be reflected quite quickly in the composition of the fillet [29]. Although conditioned to changes in the on-farm management of fish lots (namely, adopting an all-in all-out scheme), the elaboration of functional aquafeeds might well be of interest to companies that manufacture feed or food additives [30].

Particularly, *Arthrospira* sp. (Cyanobacteria) stands out not only for its high protein content (up to 70% on a dry matter basis), with an amino acid profile comparable to those

found in some reference feed proteins [31], but also for the potential to improve fillet organoleptic and nutritional characteristics in different fish species [25,32–34].

On the other hand, *Nannochloropsis* sp. is a marine microalga characterized by its richness in eicosapentaenoic acid (EPA, C20:5n3), pigments, and a variety of natural antioxidants, but also by its availability at an industrial or semi-industrial scale. These facts make this microalgal biomass a promising candidate as a commercial additive in aquafeeds. In this sense, recent results point to valuable effects of *Nannochloropsis*-based additives on qualitative parameters of aquacultured fish [10,35,36].

Both *A. platensis* and *N. gaditana* are currently authorized as feed ingredients in the E.U., according to the Feed Material Register (https://www.feedmaterialsregister.eu/(accessed on 21 May 2024). *A. platensis* is also authorized for human consumption, but not *N. gaditana*.

However, the complex and resistant cell wall (rich in cellulose) present in some microalgae genera might prevent the uptake by the animals of the bioactive substances located in the intracellular compartment of microalgae, thus hindering or reducing their potential positive effects. For this reason, it is reasonable to consider that a pre-treatment of the microalgal biomass with cellulase enzymes prior to its inclusion in feeds could facilitate the release of these bioactive compounds and thus increase their bioavailability. Indeed, it has been proven that enzymatic processing is more effective and cheaper than mechanical treatments when it comes to releasing peptides and compounds with a low molecular weight in microalgae, such as *Nannochloropsis oceanic*a, *Chlorella vulgaris*, and *Tetraselmis* [37].

With these precedents in mind, this study assesses a novel additive formulation that blends protein-rich (>50%) microalgal biomass (*Arthrospira* sp.) with another EPA-rich (up to 30% of total fatty acids) marine microalga (*Nannochloropsis* sp.), both in raw (LB-CB) and enzymatically hydrolyzed (LB-CBplus) formats, during a finishing trial carried out with fish specimens with a body size close to the commercial standard. When observed, the differences between the two additives should be attributed to the enzymatic hydrolysis, given that the starting biomass is the same and that the hydrolysates are included as a whole in the feed ingredient mix. Previous studies [10,12] have reported that the enzymatic pre-treatment increases the release of bioactive compounds from the microalgal biomass, which might explain the differences in the biological activity observed.

The results obtained indicate that growth and feed conversion were significantly higher in animals fed with the hydrolysates (LB-CBplus batch) compared to the control group, whereas animals fed with raw microalgae supplements showed values in between the other lots (Table 3). Previous studies have also reported that *A. platensis* hydrolysates included in feeds enhance seabream growth [12,38].

As mentioned previously, the functional aquafeeds impacted the proximal composition of gilthead seabream fillet (Table 4), and specifically, protein content was statistically higher in seabream supplemented with hydrolyzed microalgae biomass (LB-CBplus) compared to the non-hydrolyzed (LB-CB) and control groups. Previous studies have reported increased weight and protein deposition in the muscle of fish fed with microalgae enzymatic hydrolysates [10,13,38], a fact that has been attributed to different factors, among which the beneficial effects of microalgae on the intestinal microbiota of fish have been proposed, promoting the hydrolysis of indigestible components from the feed ingredients. Indeed, recent findings with enzymatic hydrolysates of *N. gaditana* at 5% in aquafeeds for gilthead seabream juveniles have also shown improvements in intestinal microbiota compared to a CT feed, which was free of algal biomass [39].

Lipid content was significantly lower in seabream fillets fed with algal biomass, no matter whether the biomass was pre-treated or not, indicating a greater utilization of this macronutrient by the fish while reserving muscle protein [40]. Studies on the effects of microalgae biomass in fish diets on muscle lipid composition have shown similar results [10,13,35]. The slight reduction in muscle lipid content observed in LB-CB and LB-CBplus

fillets could potentially affect the flesh quality [41], as the excess lipids can negatively influence texture.

It is well known that microalgae are a primary source of n3 PUFAs, such as EPA and DHA [42], thereby the inclusion of specific strains can increase the content of specific fatty acids in fish fillets. However, it may be more interesting to combine different microalgae species to provide a wider range of fatty acids to the fillet and thus try to obtain a better dietary quality than using a single alga, as previously suggested [43]. In our study, the dietary inclusion of microalgae-based additives increased muscle PUFAs (n3 and n6), and SFAs and MUFAs content were unchanged (Table 4). Similar findings have been reported by Liu et al. [44] and Sáez et al. [13], indicating lower monounsaturated fatty acid (MUFA) and higher PUFA contents in Atlantic salmon and gilthead seabream fed with 2.5% and 5% *N. oceanica,* and *N. gaditana*, respectively. On the other hand, Galafat et al. [12] did not observe any effect on the accretion of SFAs, MUFAs, or PUFAs in muscles after feeding with 5% and 10% *A. platensis* in gilthead seabream. Interestingly, whilst the relative content of n6 PUFA decreased, n3 PUFA content increased, particularly EPA and DHA.

On a more individualized basis, the most abundant FA in the muscle of CT fish was oleic acid (OA, 18:1n9), accounting for 25% approximately, in agreement with previous studies on this species [45], although other authors reported lower values for this monounsaturated FA (10–18%, [19]; 19–20%, [46]), possibly due to differences in feed composition. Saturated and monounsaturated fatty acids are typically constituents of triglycerides, which are the main source of energy for metabolism and growth [47].

The incorporation of microalgae into the experimental feeds caused certain modifications in the FA profile of diets (Table 2) compared to the control feed, specifically for saturated FA, oleic acid, linoleic acid, and alpha-linolenic acid, which were also reflected in the FA profile of fish fillets (Table 5). Interestingly, $\sum$PUFA-n6 and $\sum$PUFA-n3 in fillets yielded higher values in microalgae-enriched batches than those measured in the control lot, although the n3/n6 ratio did not change. In addition, some differences attributable to the enzymatic treatment were observed in fillet FA contents, and thus, LB-CBplus fillets showed higher content of LA, DHA, $\sum$PUFAs, and $\sum$n6 PUFAs than those fed with non-hydrolyzed microalgae (LB-CB). Therefore, the results suggest that the reduction in total lipid content observed in fish fed with hydrolyzed microalgae is based on the mobilization of energetic FAs, whereas structural FAs have been selectively retained in muscles. The n3/n6 ratio is an indicator of the nutritional value of fish products [48]. As mentioned, this parameter did not change among the experimental groups, even if our values were low (in the region of 1.4) compared to those described for most marine farmed fish species of around 3.0 or higher [49], likely due to the high content of plant ingredients and low inclusion of fishmeal and fish oil in commercial aquafeeds.

Other indices of lipid quality are the atherogenic index (AI) and the thrombogenic index (TI), which are determined by the relative content of various fatty acids and reflect the lipid's ability to cause aggression to the endothelium of blood vessels (atheroma formation) and produce thrombosis or embolisms, respectively [50]. The determinations of these indices are based on the functional effects of fatty acids on cholesterol metabolism. The most important hypercholesterolemic saturated fatty acids (SFAs) are C14:0 and C16:0, while the hypocholesterolemic ones are C18:1n9, C18:1n7, and PUFAs. The lower values observed for the AI in LB-CBplus fillets, together with higher values of the PUFA/SFA ratio, compared to the other two experimental batches may be attributed to certain selective retention of low-atherogenic FAs in muscles because of the bioactive effects of the substances released from microalgae owing to the enzymatic hydrolysis.

Oxidative damage is the primary cause of deterioration of fish quality after slaughtering [51], altering taste, texture, and color [52], shortening shelf life, reducing the nutritional value, and generating molecules detrimental to consumer's health [53]. Microalgae are largely acknowledged as a source of antioxidant substances [54]. In agreement, our results indicate that the inclusion of LB-CB and LB-CBplus additives markedly reduced muscle lipid oxidation (Figure 1). The dietary inclusion of *Arthrospira*

sp. and *Nannochlropsis* sp. has been assessed previously in different fish species reporting, overall, favorable impacts on muscle antioxidant status [9]. Indeed, the enzymatic hydrolysis of microalgae biomass before its incorporation into feeds has maximized this antioxidant potential, as observed in other studies as well [10,12,13,38]. This might well be attributed to the fact that enzymatic hydrolysis facilitates the release of antioxidant compounds that, otherwise, would remain less available within the intact microalgae cells [55].

The most crucial organoleptic characteristic that determines the consumer's preferences for fish in the market, and, therefore, its commercial value, is skin color [56]. It is well known that the skin tonality of farmed fish is less vivid than that from extractive fishing. Recent studies have demonstrated the interest of microalgae in the improvement of pigmentation characteristics of aquacultured fish [9,57]. Positive effects on fish skin color when *Arthrospira* sp. was added at low percentages to fish diets have been reported [38,58–61]. *N. gaditana* has also been evaluated in this regard, and thus, when included at a low level (2.5–5%) in diets, it resulted in a strong pigmentation in gilthead seabream juveniles [10,13,36], which was in agreement with its richness in pigments, such as chlorophylls, β-carotene, violaxanthin, and vaucheriaxanthin [62].

Our results indicated that the LB-CB and LC-CBplus additives intensified yellowness (b* parameter) and greenish (a*) tonalities in gilthead seabream skin (Figure 2), which contributed to an enhanced "visual quality" of the skin of fish, a crucial aspect for the commercial value of fillets. The greener pigmentation owing to microalgae has been attributed to chlorophyll [63], taking into consideration that fish are not able to synthesize their pigments but instead must incorporate them through the feed [64].

On the other hand, when it comes to flesh color, the functional additives and storage time caused a certain impact on the parameters measured, although less markedly than described for the skin side (Figure 2). Of note was the increase in the b* parameter (Figure 3.C), especially in CT fillets after 7 *dpm*. In the literature, this rise has been attributed to the accumulation of lipid oxidation products and free amino groups from proteins [65], which leads to yellowish tonality. On the contrary, the increase in b* was markedly delayed in fish fed with microalgae-based additives, especially when algae biomass was pre-treated (LB-CBplus). Interestingly, the persistence of differences throughout the cold storage period compared to CT roughly mirrored the results observed for fillet lipid oxidation (Figure 3A), thereby confirming the valuable effects of functional additives on quality parameters.

The possibility of favorably modifying the pigmentation of the skin of commercial fish (but without altering the pigmentation of the muscle) by feeding them with microalgae is very promising from a practical point of view. This approach represents a strategy to improve one of the most criticized aspects of aquaculture fish compared to wild fish, namely, poor skin pigmentation.

In addition to color, the textural attributes of fish muscles are crucial in terms of consumer acceptance [57]. The muscle constitutes the primary component of the edible fraction of commercial fish, representing up to 60–70% of total body weight. Consequently, considerable attention has been paid to understanding the impact of nutrition on the properties of fish muscles across various commercial species. As previously highlighted, the structure of the muscle influences the sensory properties of the fillet, including its texture [66].

Regarding the hardness parameter, initial values were significantly higher in LB-CBplus and LB-CB fillets than in the CT lot (Figure 4), and these differences persisted during the initial 5 days of cold storage. This same effect was observed for the gumminess and chewiness parameters.

These data indicate that the inclusion of *A. platensis* and *N. gaditana* in the experimental aquafeeds increased the consistency of the fish muscle, a fact that likely might improve the attractiveness of fillets for consumers. The textural characteristics of the muscle depend mainly on myofibrillar proteins and collagen content, as they are the

major components of the fish muscle; the greater the degradation of these components, the lower the firmness [67]. However, muscle lipid content also plays a role in hardness [68], and thus a higher hardness might also be related to the lower content of muscle lipids found in those fillets (Table 4).

Even if the literature on the influence of dietary microalgae on the textural parameters of fish and other organoleptic characteristics is scarce, some authors have shown that these parameters have a positive influence. For example, aquafeed supplementation with *A. platensis* resulted in firmer fillets in ayu specimens (*Plecoglossus altivelis;* [32]), porgy (*Pargus major;* [33]), and goldlined seabream (*Rhabdosargus sarba;* [69]). Similarly, Liao et al. [70] and Watanabe et al. [71,72] also reported that feeds enriched with *A. platensis* (5% inclusion level) decreased muscle lipid content and improved the texture of golden yellowtail (*Pseudocaranx dentex*). Additionally, an increase in firmness coupled with a decrease in lipid content of gilthead seabream fillets was observed by Sáez et al. [10] following the administration of a finishing diet enriched with both raw and enzymatically hydrolyzed *N. gaditana*.

The effects of the microalgae-based additives on pH and water holding capacity (Figure 5) of seabream fillets were less evident than those observed for firmness. Values for pH tended to be lower in microalgae-enriched batches at any sampling point, although significant differences were observed at 5 and 9 *dpm*. Values were, however, within the range of acceptability for fresh fish (6.3–6.4, [73]) in all cases. Changes in fish muscle pH are typically characterized by an initial decrease, caused by the post-mortem synthesis of lactic acid due to the degradation of muscle glycogen through anaerobic pathways [73], which is determined by the nutritional status of the fish at the time of slaughtering. Subsequently, an increase in this parameter due to the production of alkaline substances can be observed, owing to both muscle cell autolysis and microbial hydrolysis of muscle proteins [74]. This pattern of initial decrease and further increase in pH has also been observed in our study, but roughly, no clear effect of the dietary supplementation with microalgae-based additives could be observed.

WHC, which represents the percentage of water retained in the muscle, decreases as storage time increases, which is in agreement with the phenomenon of protein degradation [75]. However, this parameter was not influenced by the dietary treatments (Figure 5B). Similar results were observed for seabream fillets when low inclusion levels (2.5 and 5%) of *N. gaditana* were incorporated into aquafeeds [10].

Despite the beneficial effects described above, several limitations of this study cannot be obviated; on the one hand, there are limitations related to the heterogeneity of the algal biomass, which is determined by differences among species and the effect of cultivation conditions on its composition. On the other hand, a cost/benefit study of the inclusion of this microalgal biomass should be carried out for each specific case, given the high cost of this material on the market. Finally, it is also necessary to evaluate the possible effects of microalgal biomass on the subjective organoleptic parameters perceived by consumers, for instance, by means of blind taste tests. All these aspects need to be addressed before any practical application in commercial fish farms might be recommended.

5. Conclusions

Microalgae are rich in a wide variety of bioactive substances and are potentially interesting due to the quality characteristics of fish fillets, and even at low inclusion levels, numerous studies have provided evidence of positive effects on the objective quality parameters of fish. Our results agree with the previous literature, indicating that the inclusion of two commercial additives, LB-CB and LB-CBplus, based on the microalgae *N. gaditana* and the cyanobacterium *A. platensis*, either raw or enzymatically hydrolyzed, were able to improve some valuable parameters that influence consumer acceptability, and they also enhanced certain nutritional aspects of the edible fraction of fish.

In this regard, it is known that the dullness of the pigmentation of farmed fish is a clear disadvantage in terms of consumer preference compared to wild fish. According to

the results, the inclusion of both additives was able to improve skin tonality, especially regarding yellow and green colors, so that the fillets look more similar to those obtained from wild-caught fish. In addition to the importance of skin color, feeds enriched with both additives also enhanced textural parameters up to 5 *dpm*, especially when using pre-treated microalgae (LB-CBplus).

Furthermore, although not related to organoleptic properties, both additives were also able to protect muscle lipids from oxidation during cold storage, and this effect was more noticeable when the microalgal biomass was enzymatically hydrolyzed.

These favorable effects, together with some improvement in fillet composition (higher protein and lower total lipid contents, with selective retention of PUFAs), have proved the interest of microalgae in finishing diets for gilthead seabream. However, there are still several limitations that need to be addressed before microalgal-based additives can be extended for practical use in fish farms.

Institutional Review Board Statement: Gilthead seabream (*Sparus aurata*) juveniles were provided by a commercial firm (CUPIBAR, Chiclana de la Frontera, Cádiz). The feeding trial was carried out at Servicios Centrales de Investigacion en Cultivos Marinos (SCI-CM, CASEM, Universidad de Cadiz, Spain). All experimental procedures complied with the Guidelines of the European Union (Directive 2010/63/EU) and the Spanish regulations (Real Decreto 53/2013, as amended by RD 118/2021) on the protection of laboratory animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: M.I. Sáez, F.J. Alarcón and T.F Martínez are partners in the spin-off 'LifeBioencapsulation, S.L.', which has developed the additives used in this work. They declare that this circumstance has not interfered in any way with the research carried out in this study.

References

1. FAO. *The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation*; FAO: Rome, Italy 2023. https://doi.org/10.4060/cc0461en.
2. Dawood, M.A.; Koshio, S.; Esteban, M.A. Beneficial roles of feed additives as immunostimulants in aquaculture: A review. *Rev. Aquac.* **2018**, *10*, 950–974. https://doi.org/10.1111/raq.12209.
3. Ghosh, S.; Sarkar, T.; Pati, S.; Kari, Z.A.; Edinur, H.A.; Chakraborty, R. Novel bioactive compounds from marine sources as a tool for functional food development. *Front. Mar. Sci.* **2022**, *9*, 10–3389. https://doi.org/10.3389/fmars.2022.832957.
4. Shah, M.R.; Lutzu, G.A.; Alam, A.; Sarker, P.; Chowdhury, M.K.; Parsaeimehr, A.; Liang, Y.; Daroch, M. Microalgae in aquafeeds for a sustainable aquaculture industry. *J. Appl. Phycol.* **2018**, *30*, 197–213. http://doi.org/10.1007/s10811-017-1234-z.
5. Siddik, M.A.; Sørensen, M.; Islam, S.M.; Saha, N.; Rahman, M.A.; Francis, D.S. Expanded utilisation of microalgae in global aquafeeds. *Rev. Aquac.* **2024**, *16*, 6–33. https://doi.org/10.1111/raq.12818.
6. Magnoni, L.J.; Martos-Sitcha, J.A.; Queiroz, A.; Calduch-Giner, J.A.; Gonçalves, J.F.; Rocha, C.M.R.; Abreu, H.T.; Schrama, J.W.; Ozorio, R.O.A.; Pérez-Sánchez, J. Dietary supplementation of heat-treated *Gracilaria* and *Ulva* seaweeds enhanced acute hypoxia tolerance in gilthead seabream (*Sparus aurata*). *Biol. Open.* **2017**, *6*, 897–908. https://doi org/10 1242/bio024299.
7. Matos, E.; Dias, J.; Dinis, M.T.; Silva, T.S. Sustainability vs. quality in in gilthead seabream (*Sparus aurata* L.) farming: Are trade-offs inevitable? *Rev. Aquac.* **2017**, *9*, 388–409. https://doi org/10.1111/raq.12144.

8. Ambati, R.R.; Siew Moi, P.; Ravi, S.; Aswathanarayana, R.G. Astaxanthin: Sources, extraction, stability, biological activities and its commercial applications—A review. *Mar. Drugs.* **2014**, *12*, 128–152. https://doi.org/10.3390/md12010128.

9. Vizcaíno, A.J.; Sáez, M.I.; Galafat, A.; Martínez, T.F.; Alarcón, F.J. Chapter 10—Practical approach to the use of microalgae in aquaculture feeds. In *Sustainable Industrial Processes Based on Microalgae*; Lafarga, T., Acién, G., Eds.; Elsevier: Amsterdam, The Netherlands, 2024; pp. 209–233. https://doi.org/10.1016/B978-0-443-19213-5.00010-8.

10. Sáez, M.I.; Galafat, A.; Suárez, M.D.; Chaves-Pozo, E.; Arizcun, M.; Ayala, M.D.; Alarcón, F.J.; Martínez, T.F. Effects of raw and hydrolysed *Nannochloropsis gaditana* biomass included at low level in finishing diets for gilthead seabream (*Sparus aurata*) on fillet quality and shelf life. *J. Appl. Phycol.* **2023**, *35*, 1163–1181. https://doi.org/10.21203/rs.3.rs-2284166/v1.

11. Sheikhzadeh, N.; Soltani, M.; Heidarieh, M.; Ghorbani, M. Role of dietary microalgae on fish health and fillet quality: Recent insights and future prospects. *Fishes* **2024**, *9*, 26. https://doi.org/10.3390/fishes9010026.

12. Galafat, A.; Vizcaíno, A.J.; Sáez, M.I.; Martínez, T.F.; Arizcun, M.; Chaves-Pozo, E.; Alarcón, F.J. Assessment of dietary inclusion of crude or hydrolysed *Arthrospira platensis* biomass in starter diets for gilthead seabream (*Sparus aurata*). *Aquaculture* **2022**, *548*, 737680. http://doi.org/10.1016/j.aquaculture.2021.737680.

13. Sáez, M.I.; Galafat, A.; Vizcaíno, A.J.; Chaves-Pozo, E.; Ayala, M.D.; Arizcun, M.; Alarcón, F.J.; Suárez, M.D.; Martínez, T.F. Evaluation of *Nannochloropsis gaditana* raw and hydrolysed biomass at low inclusion level as dietary functional additive for gilthead seabream (*Sparus aurata*) juveniles. *Aquaculture.* **2022**, *556*, 738288. https://doi.org/10.1016/j.aquaculture.2022.738288.

14. Molina-Roque, L.; Bárany, A.; Sáez, M.I.; Alarcón, F.J.; Tapia, S.T.; Fuentes, J.; Mancera, J.M.; Perera, E.; Martos-Sitcha, J.A. Biotechnological treatment of microalgae enhances growth performance, hepatic carbohydrate metabolism and intestinal physiology in gilthead seabream (*Sparus aurata*) juveniles close to commercial size. *Aquac. Rep.* **2022**, *25*, 101248. https://doi.org/10.1016/j.aqrep.2022.10124.

15. AOAC. *Official Methods of Analysis of AOAC International*, 17th ed.; Association of Analytical Communities: Gaithersburg, MD, USA, 2000.

16. Folch, J. Simple method for the isolation and purification of total lipids form animal tissue. *J. Biol. Chem.* **1957**, *226*, 497–509. http://doi.org/10.1016/S0021-9258(18)64849-5.

17. Rodríguez-Ruiz, J.; Belarbi, E.H.; Sánchez, J.L.G.; Alonso, D.L. Rapid simultaneous lipid extraction and transesterification for fatty acid analyses. *Biotech. Tech.* **1998**, *12*, 689–691.

18. Lepage, G.; Roy, C.C. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *J. Lipid Res.* **1984**, *25*, 1391–1396. https://doi.org/10.1016/S0022-2275(20)34457-6.

19. Senso, L.; Suarez, M.D.; Ruiz-Cara, T.; Garc a-Gallego, M. The possible effects of harvesting season and chilled storage on the fatty acid profile of the fillet of farmed gilthead sea bream (*Sparus aurata*). *Food Chem.* **2007**, *101*, 298–307. https://doi.org/10.1016/j.foodchem.2006.01.036.

20. Buege, J.A.; Aust, S.D. Microsomal lipid peroxidation. *Methods Enzymol.* **1978**, *52*, 302–310. https://doi.org/10.1016/s0076-6879(78)52032-6.

21. CIE. *Colorimetry Commission International de l'Eclairage*, 2nd ed.; Publication CIE 15 2 Vienna; CIE: Cambridge, UK, 1986.

22. Bourne, M.C. Texture Profile analysis. *Food Tech.* **1978**, *32*, 62–66.

23. Suárez, M.D.; Martínez, T.F.; Sáez, M.I.; Morales, A.E.; García-Gallego, M. Effects of dietary restriction on *post-mortem* changes in white muscle of sea bream (*Sparus aurata*). *Aquaculture* **2010**, *307*, 49–55. https://doi.org/10.1016/j.aquaculture.2010.07.006.

24. Tongsiri, S.; Mang-Amphan, K.; Peerapornpisal, Y. Effect of replacing fishmeal with *Spirulina* on growth, carcass composition and pigment of the Mekong giant catfish. *Asian J. Agric. Sci.* **2010**, *2*, 106–110.

25. Teimouri, M.; Amirkolaie, A.K.; Yeganeh, S. The effects of *Spirulina platensis* meal as a feed supplement on growth performance and pigmentation of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **2013**, *396*, 14–19. https://doi.org/10.1016/j.aquaculture.2013.02.009.

26. Roy, S.S.; Pal, R. Microalgae in aquaculture: A review with special references to nutritional value and fish dietetics. In *Proceedings of the Zoological Society*; Springer: Delhi, India, 2015; Volume 68, pp. 1–8. https://doi.org/10.1007/s12595-013-0089-9.

27. Amer, S. Effect of *Spirulina platensis* as feed supplement on growth performance, immune response and antioxidant status of mono-sex Nile Tilapia (*Oreochromis niloticus*). *Benha Vet. Med. J.* **2016**, *30*, 1–10. https://doi.org/10.21608/BVMJ.2016.31332.

28. Kousoulaki, K.; Mørkøre, T.; Nengas, I.; Berge, R.K.; Sweetman, J. Microalgae and organic minerals enhance lipid retention efficiency and fillet quality in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **2016**, *451*, 47–57. https://doi.org/10.1016/j.aquaculture.2015.08.027.

29. Sáez, M.I.; Suárez, M.D.; Cárdenas, S.; Martínez, T.F. Influence of freezing and freezing-thawing cycles on textural and biochemical changes of meagre (*Argyrosomus regius* L) fillets during further cold storage. *Int. J. Food Prop.* **2014**, *18*, 1635–1647. https://doi.org/10.1080/10942912.2014.919319.

30. Batista, S.; Pintado, M.; Marques, A.; Abreu, H.; Silva, J.L.; Jessen, F.; Tulli, F.; Valente, L.M.P. Use of technological processing of seaweed and microalgae as strategy to improve their apparent digestibility coefficients in European seabass (*Dicentrarchus labrax*) juveniles *J. Appl. Phycol.* **2020**, *32*, 3429–3446. https://doi.org/10.1007/s10811-020-02185-2.

31. Becker, E.W. Micro-algae as a source of protein. *Biotechnol. Adv.* **2007**, *25*, 207–210. https://doi.org/10.1016/j.biotechadv.2006.11.002.

32. Mori, T.; Muranaka, T.; Miki, W.; Yamaguchi, K.; Konosu, S.; Watanabe, T. Pigmentation of cultured sweet smelt fed diets supplemented with a blue-green alga *Spirulina maxima*. *Nippon Suisan Gakk.* **1987**, *53*, 433–438. https://doi.org/10.2331/suisan.53.433.

33. Mustafa, M.G.; Umino, T.; Nakagawa, H. The effect of *Spirulina* feeding on muscle protein deposition in red sea bream, *Pagrus major*. *J. Appl. Ichthyol.* **1994**, *10*, 141–145. https://doi.org/10.1111/j.1439-0426.1994.tb00153.x.

34. Teimouri, M.; Yeganeh, S.; Amirkolaie, A.K. The effects of *Spirulina platensis* meal on proximate composition, fatty acid profile and lipid peroxidation of rainbow trout (*Oncorhynchus mykiss*) muscle. *Aquac. Nutr.* **2016**, *22*, 559–566. https://doi.org/10.1111/anu.12281.

35. Qiao, H.; Hu, D.; Ma, J.; Wang, X.; Wu, H.; Wang, J. Feeding effects of the microalga *Nannochloropsis* sp. on juvenile turbot (*Scophthalmus maximus* L.). *Algal Res.* **2019**, *41*, 101540. https://doi.org/10.1016/j.algal.2019.101540.

36. Sales, R.; Galafat, A.; Vizcaíno, A.J.; Sáez, M.I.; Martínez, T.F.; Cerón-García, M.C.; Alarcón, F.J. Effects of dietary use of two lipid extracts from the microalgae *Nannochloropsis gaditana* (Lubián, 1982) alone and in combination on growth and muscle composition in juvenile gilthead seabream, *Sparus aurata*. *Algal Res.* **2021**, *10*, 2270. https://doi.org/10.1016/j.algal.2020.102162.

37. Agboola, J.O.; Teuling, E.; Wierenga, P.A.; Gruppen, H.; Schrama, J.W. Cell wall disruption: An effective strategy to improve the nutritive quality of microalgae in African catfish (*Clarias gariepinus*). *Aquac. Nutr.* **2019**, *25*, 783–797. https://doi.org/10.1111/anu.12896.

38. Galafat, A.; Vizcaíno, A.J.; Sáez, M.I.; Martínez, T.F.; Jérez-Cepa, I.; Mancera, J.M.; Alarcón, F.J. Evaluation of *Arthrospira* sp. enzyme hydrolysate as dietary additive in gilthead seabream (*Sparus aurata*) juveniles. *J. Appl. Phycol.* **2020**, *32*, 3089–3100. https://doi.org/10.1007/s10811-020-02141-0.

39. Cerezo-Ortega, I.M.; Di Zeo-Sánchez, D.E.; García-Márquez, J.; Ruiz-Jarabo, I.; Sáez-Casado, M.I.; Balebona, M.C.; Moriñigo, M.A.; Tapia-Paniagua, S.T. Microbiota composition and intestinal integrity remain unaltered after the inclusion of hydrolysed *Nannochloropsis gaditana* in *Sparus aurata* diet. *Sci Rep.* **2021**, *11*, 18779. https://doi.org/10.1038/s41598-021-98087-5.

40. James, R.; Sampath, K.; Thangarathinam, R.; Vasudevan, I. Effects of dietary Spirulina level on growth, fertility, coloration and leucocyte count in red swordtail, *Xiphophorus helleri*. *Isr. J. Aquac. -Bamidgeh* **2006**, *58*, 97–104. https://doi.org/10.46989/001c.20433.

41. Grigorakis, K. Compositional and organoleptic quality of farmed and wild gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) and factors affecting it: A review. *Aquaculture* **2007**, *272*, 55–75. https://doi.org/10.1016/j.aquaculture.2007.04.062.

42. Gong, Y.; Bandara, T.; Huntley, M.; Johnson, Z.I.; Días, J.; Dahle, D.; Sørensen, M.; Kiron, V. Microalgae *Scenedesmus* sp. as a potential ingredient in low fishmeal diets for Atlantic salmon (*Salmo salar* L.). *Aquaculture* **2019**, *501*, 455–464. https://doi.org/10.1016/j.aquaculture.2018.11.049.

43. Harel, M.; Koven, W.; Lein, I.; Bar, Y.; Behrens, P.; Stubblefield, J.; Zohar, J.; Place, A.R. Advanced DHA, EPA and ArA enrichment materials for marine aquaculture using single cell heterotrophs. *Aquaculture* **2002**, *213*, 347–362. https://doi.org/10.1016/S0044-8486(02)00047-9.

44. Liu, C.; Palihawadana, A.M.; Nadanasabesan, N.; Vasanth, G.K.; Vatsos, I.N.; Días, J.; Valente, L.M.P.; Micallef, G.; Sørensen, M.; Kiron, V. Utilization of *Nannochloropsis oceanica* in plant-based feeds by Atlantic salmon (*Salmo salar*). *Aquaculture* **2022**, *561*, 738651. https://doi.org/10.1016/j.aquaculture.2022.738651.

45. Grigorakis, K.; Alexis, M.N.; Taylor, A.; Hole, M. Comparison of wild and cultured gilthead sea bream, (*Sparus aurata*): Composition, appearance and seasonal variations. *Int. J. Food Sci. Tech.* **2002**, *37*, 477–484. https://doi.org/10.1046/j.1365-2621.2002.00604.x.

46. Izquierdo, M.S.; Montero, D.; Robaina, L.; Caballero, M.J.; Rosenlund, G.; Gines, R. Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. *Aquaculture* **2005**, *250*, 431–444. https://doi.org/10.1016/j.aquaculture.2004.12.001.

47. Rodríguez, C.; Acosta, C.; Badía, P.; Cejas, J.R.; Santamaría, F.J.; Lorenzo, A. Assessment of lipid and essential fatty acids requirements of black seabream (*Spondyliosoma cantharus*) by comparison of lipid composition in muscle and liver of wild and captive adult fish. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **2004**, *139*, 619–629. https://doi.org/10.1016/j.cbpc.2004.07.013.

48. Hu, F.B.; Bronner, L.; Willett, W.C.; Stampfer, M.J.; Rexrode, K.M.; Christine, M.; Albert, C.M.; Hunter, D.; Manson, J.E. Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA* **2002**, *287*, 1815–1821. https://doi.org/10.1001/jama.287.14.1815.

49. Celik, M. Seasonal changes in the proximate chemical compositions and fatty acids of chub mackerel (*Scomber japonicus*) andhorse mackerel (*Trachurus trachurus*) from the northeastern Mediterranean Sea. *Int. J. Food Sci. Technol.* **2008**, *43*, 933–938. https://doi.org/10.1111/j.1365-2621.2007.01549.x.

50. Ghaeni, M.; Ghahfarokhi, K.N.; Zaheri, L. Fatty acids profile, Atherogenic (IA) and thrombogenic (IT) health lipid indices in *Leiognathusbindus* and *Upeneussulphureus*. *J. Mar. Sci. Res. Dev.* **2013**, *3*, 138. https://doi.org/10.4172/2155-9910.1000138.

51. Archile-Contreras, A.C.; Purslow, P.P. Oxidative stress may affect meat quality by interfering with collagen turnover by muscle fibroblasts. *Food Res. Int.* **2011**, *44*, 582–588. https://doi.org/10.1016/j.foodres.2010.12.002.

52. Hosseini, S.V.; Abedian-Kenari, A.; Rezaei, M.; Nazari, R.M. Influence of the in vivo addition of alpha-tocopherol acetate with three lipid sources on the lipid oxidation and fatty acid composition of Beluga sturgeon, *Huso huso*, during frozen storage. *Food Chem.* **2010**, *118*, 341–348. https://doi.org/10.1016/j.foodchem.2009.04.131.

53. Secci, G.; Parisi, G. From farm to fork: Lipid oxidation in fish products. A review. *Ital. J. Anim. Sci.* **2016**, *15*, 124–136. https://doi.org/10.1080/1828051X.2015.1128687.

54. Almendinger, M.; Saalfrank, F.; Rohn, S.; Kurth, E.; Springer, M.; Pleissner, D. Characterization of selected microalgae and cyanobacteria as sources of compounds with antioxidant capacity. *Algal Res.* **2021**, *53*, 102168. https://doi.org/10.1016/j.algal.2020.102168.

55. Liu, C.; Liu, H.; Xu, W.; Han, D.; Xie, S.; Jin, J.; Zhu, X. Effects of dietary *Arthrospira platensis* supplementation on the growth, pigmentation, and antioxidation in yellow catfish (*Pelteobagrus fulvidraco*). *Aquaculture* **2019**, *510*, 267–275. https://doi.org/10.1016/j.aquaculture.2019.05.067.

56. Sefc, K.M.; Brown, A.C.; Clotfelter, E.D. Carotenoid-based coloration in cichlid fishes. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2014**, *173*, 42–51. https://doi.org/10.1016/j.cbpa.2014.03.006.

57. Nagappan, S.; Das, P.; AbdulQuadir, M.; Thaher, M.; Khan, S.; Mahata, C.; Kumar, G. Potential of microalgae as a sustainable feed ingredient for aquaculture. *J. Biotechnol.* **2021**, *341*, 1–20. https://doi.org/10.1016/j.jbiotec.2021.09.003.

58. Sun, X.; Chang, Y.; Ye, Y.; Ma, Z.; Liang, Y.; Li, T.; Luo, L. The effect of dietary pigments on the coloration of Japanese ornamental carp koi (*Cyprinus carpio* L.). *Aquaculture* **2012**, *342*, 62–68. https://doi.org/10.1016/j.aquaculture.2012.02.019.

59. Ansarifard, F.; Rajabi Islami, H.; Shamsaie Mehrjan, M.; Soltani, M. Effects of *Arthrospira platensis* on growth, skin color and digestive enzymes of Koi, *Cyprinus carpio*. *Iran. J. Fish. Sci.* **2018**, *17*, 381–393. https://doi.org/10.22092/IJFS.2018.115878.

60. Dineshbabu, G.; Goswami, G.; Kumar, R.; Sinha, A.; Das, D. Microalgae–nutritious, sustainable aqua-and animal feed source. *J. Funct. Foods*. **2019**, *62*, 103545. https://doi.org/10.1016/j.jff.2019.103545.

61. Liu, C.; Li, Y.; Chen, Z.; Yuan, L.; Liu, H.; Han, D.; Xie, S. Effects of dietary whole and defatted *Arthrospira platensis* (Cyanobacterium) on growth, body composition and pigmentation of the yellow catfish *Pelteobagrus fulvidraco*. *J. Appl. Phycol.* **2021**, *33*, 2251–2259. https://doi.org/10.1007/s10811-021-02445-9.

62. Cerón-García, M.C.; González-López, C.; Camacho-Rodríguez, J.; López-Rosales, L.; García-Camacho, F.; Molina-Grima, E. Maximizing carotenoid extraction from microalgae used as food additives and determined by liquid chromatography (HPLC). *Food Chem.* **2018**, *257*, 316–324. https://doi.org/10.1016/j.foodchem.2018.02.154.

63. Cardinaletti, G.; Messina, M.; Bruno, M.; Tulli, F.; Poli, B.M.; Giorgi, G.; Chini-Zittelli, G.; Tredici, M.; Tibaldi, E. Effects of graded levels of a blend of Tisochrysis lutea and Tetraselmis suecica dried biomass on growth and muscle tissue composition of European sea bass (*Dicentrarchus labrax*) fed diets low in fish meal and oil. *Aquaculture* **2018**, *85*, 173–182. https://doi.org/10.1016/j.aquaculture.2017.11.049.

64. Mukherjee, P.; Nandi, C.; Khatoon, N.; Pal, R.; Pal, R. Mixed algal diet for skin colour enhancement of ornamental fishes. *J. Algal Biomass Util*. **2015**, *6*, 35–46.

65. Silva-Brito, F.; Alexandrino, D.A.M.; Jia, Z.; Mo, Y.; Kijjoa, A.; Abreu, H.; Carvalho, M.F.; Ozorio, R.; Magnoni, L. Fish performance, intestinal bacterial community, digestive function and skin and fillet attributes during cold storage of gilthead seabream (*Sparus aurata*) fed diets supplemented with *Gracilaria* by-products. *Aquaculture* **2021**, *541*, 736808. https://doi.org/10.1016/j.aquaculture.2021.736808.

66. Ayala, M.D.; Galián, C.; Fernández, V.; Chaves-Pozo, E.; García de la Serrana, D.; Sáez, M.I.; Galafat, A.; Alarcón, F.J.; Martínez, T.F.; Arizcun, M. Influence of low dietary inclusion of the microalga *Nannochloropsis gaditana* (Lubián 1982) on performance, fish morphology, and muscle growth in juvenile gilthead seabream (*Sparus aurata*). *Animals* **2020**, *10*, 2270. https://doi.org/10.3390/ani10122270.

67. Cheng, J.-H.; Qu, J.-H.; Sun, D.-W.; Zeng, X.-A. Texture and structure measurements and analyses for evaluation of fish and fillet freshness quality: A review. *Compr. Rev. Food Sci. Food Saf.* **2014**, *9*, 52–61. https://doi.org/10.1111/1541-4337.12043.

68. Lefevre, F.; Cardinal, M.; Bugeon, J.; Labbe, L.; Medale, F.; Quillet, E. Selection for muscle fat content and triploidy affect flesh quality in pan-size rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* **2015**, *448*, 569–577. https://doi.org/10.1016/j.aquaculture.2015.06.029.

69. El-Sayed, A.M. Evaluation of soybean meal, spirulina meal and chicken offtal meal as protein sources for silver seabream (*Rhabdosargus sarba*) fingerlings. *Aquaculture* **1994**, *127*, 169–176. https://doi.org/10.1016/0044-8486(94)90423-5.

70. Liao, W.L.; Takeuchi, T.; Watanabe, T.; Yamaguchi, K. Effect of dietary Spirulina supplementation on extractive nitrogenous constituents and sensory test of cultured striped jack. *J. Tokyo Univ. Fish*. **1990**, *77*, 241–246.

71. Watanabe, T.; Liao, W.L.; Takeuchi, T.; Yamamoto, H. Effect of dietary Spirulina supplementation on growth performance and flesh lipids of cultured striped jack. *J. Tokyo Univ. Fish.* **1990**, *77*, 231–239.

72. Watanabe, T.; Takeuchi, T.; Okamoto, N.; Satoh, S.; Iso, N.; Sakamoto, H.; Arakawa, T. Improvement of flesh quality of cultured striped jack with a newly developed soft-dry pellet. *J. Tokyo Univ. Fish.* **1993**, *80*, 19–29.

73. Huss, H.H. *Quality and Quality Changes in Fresh Fish*; FAO Fisheries Technical Paper No. 348; Food and Agriculture Organization (FAO) of the United Nations: Rome, Italy, 1995.

74. Goulas, A.E.; Kontominas, M.G. Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (*Sparus aurata*): Biochemical and sensory attributes. *Food Chem.* **2007**, *100*, 287–296. https://doi.org/10.1016/j.foodchem.2005.09.045.

75. Duun, A.S.; Rustad, T. Quality changes during superchilled storage of cod (*Gadus morhua*) fillets. *Food Chem.* **2007**, *105*, 1067–1107. https://doi.org/10.1016/j.foodchem.2007.05.020.