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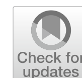
RESEARCH



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

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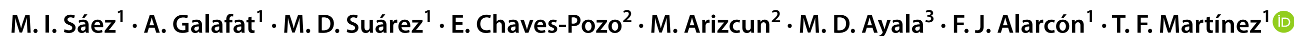

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Abstract

Numerous studies evaluating the effects of the incorporation of microalgae in feeds have reported favourable impacts on different physiological aspects of aquacultured fish. Although productivity is the major goal in terms of profitability in fish farming, qualitative aspects are gaining the attention of producers, given the relevance of quality attributes related to organoleptic parameters, proximal composition, and shelf life on the commercial value of fish. Indeed, microalgae are acknowledged for their richness in substances with potential positive effects on all those quality attributes. In this context, this study assesses the effects of finishing diets enriched with the microalga *Nannochloropsis gaditana*, either crude or enzymatically hydrolysed, on several quality parameters of gilthead seabream (*Sparus aurata*) fillets. Two inclusion levels (2.5 and 5%) of raw and enzymatically hydrolysed microalgal biomass were incorporated into diets, plus a microalgae-free control diet, and a 42-day feeding trial was carried out on fish of commercial size (approx. 500 g body weight). The influence of the experimental diets on fish biometry, fillet quality parameters, and shelf life was evaluated. The results indicate, overall, that microalgae-enriched diets yielded favourable, dose-dependent effects on several objective quality parameters of fillets, namely, improved fatty acid profile, reduced microbial counts, enhanced lipid oxidative status, and improved textural and skin colour attributes. Although the enzymatic pre-treatment of the microalgal biomass was expected to impact positively its functional effects on all quality parameters, however, no general trend was observed.

Keywords Fillet quality · Microalgae hydrolysis · Finishing diets · Fish shelf life · Functional additives

Introduction

The interest in microalgae as an ingredient in fish feeds is increasing in the last years. Studies have been carried out evaluating the effects of the incorporation of microalgae in aquafeeds at levels up to 15%, and favourable results have been reported pointing to enhanced growth and feed efficiency (Teimouri et al. 2013a; Vizcaíno et al. 2018; Wei et al. 2021), as well as improved stress response and resistance to infectious diseases (Cerezuela et al. 2012). Potent antioxidant, antimicrobial and immunostimulant effects have been confirmed in fish (Watanuki et al. 2006) and also the ability to mitigate the adverse effects caused by the inclusion of plant-based ingredients in feeds (Bravo-Tello et al. 2017).

Beyond productivity in quantitative terms, current aquaculture is also increasingly aware of the relevance of many other aspects that shape the concept of quality fish for human consumption. In this regard, the impact of feeding strategies on the organoleptic attributes of fish is becoming more and more relevant (Matos et al. 2017), owing to the influence of the external appearance on consumer purchasing decision. For instance, farmed fish specimens frequently lack the vivid coloration and gloss characteristics of wild individuals, this decreasing consumer acceptance and market value.

Microalgae are reputed to yield positive effects on fish colour parameters (Gouveia et al. 2002; Teimouri et al. 2013a, b; Cardinaletti et al. 2018), since they are rich in

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substances with recognised effects in this respect, such as carotenoids, chlorophylls and xanthophylls. Not only colour, but also textural parameters are crucial for fillet quality, which worsen rapidly owing to spoilage processes (Matos et al. 2017) under cold storage. Studies addressing the influence of the dietary inclusion of microalgae on the texture of commercial fish are very scarce, although some authors have observed improved firmness, gaping decrease, and overall, enhanced fillet quality attributes (Watanabe 1990; Kousoulaki et al. 2016). To our knowledge, no reports are available addressing this topic in gilthead seabream (*Sparus aurata*).

In addition to organoleptic parameters, the excellent nutritional quality of lipids is a typical attribute of fish for human consumption, as it is a major source of long chain n-3 polyunsaturated fatty acid (n-3 PUFA). However, the continuous search for substitution of fishmeal and fish oil by plant ingredients might jeopardise the qualitative composition of fatty acids in aquacultured fish. The ability of marine fish to synthesize n-3 PUFA is very limited, almost negligible compared to the role of the diet composition on the final profile of fatty acids of fish muscle. Microalgal biomass is a valuable source of n-3 PUFA (especially EPA, 20:5n-3, and DHA, 22:6n-3), given that microalgae are the most important primary producers of such PUFAs in marine trophic systems. Some authors have described selective retention of n-3 PUFAs in muscle of fish fed with microalgae-supplemented diets (Tibaldi et al. 2015; Cardinaletti et al. 2018; He et al. 2018).

Oxidative damage that begins after slaughter affects fish quality (Archile-Contreras and Purslow 2011) by altering flavour, texture and colour (Hosseini et al. 2010), shortening commercial shelf life, decreasing the nutritional value, and generating molecules with potentially harmful effects for humans (Secci and Parisi 2016). The incorporation of different species of microalgae in fish feeds has shown positive effects on the antioxidant capacity and prevention of oxidative stress in several fish species (Teimouri et al. 2019; Sales et al. 2021).

Some microalgae species have strong cell walls that constitute a hindrance to the extraction of inner compounds, which might well decrease their bioavailability and digestibility (Niccolai et al. 2019). Namely, *N. gaditana* has a thick cell wall rich in cellulose (Scholz et al. 2014), which might well hamper practical utilization of this species in aquafeeds, as suggested previously (Sáez et al. 2022). Consequently, several strategies have been explored aimed at disrupting microalgae cell walls, which roughly can be grouped in physical and enzymatic (Agboola et al. 2019; Batista et al. 2020; Timira et al. 2022). The enzymatic hydrolysis of microalgal cell walls promotes the release of intracellular components (Almendinger et al. 2021), and therefore increases nutrient availability and digestibility of algae by fish (Teuling et al. 2019).

Although previous studies have reported favourable effects of *N. gaditana*-enriched diets on skin colour and lipid oxidation parameters, however, they were carried on at early stages of the production cycle (Sáez et al. 2022), far from fish of commercial size. To our knowledge, the assessment of the possible influence of finishing diets enriched with *N. gaditana*, either crude or enzymatically hydrolysed, on muscle quality parameters of gilthead seabream (*S. aurata*) remains to be ascertained. In this regard, this study is aimed at evaluating the effects of two inclusion levels (2.5 and 5%) of raw and enzymatically hydrolysed *N. gaditana* biomass in finishing diets for gilthead seabream. The possible influence of the experimental diets kept for a 42-day period on fish biometry, fillet quality and shelf life were assessed, and for this purpose, parameters related to microbial counts, proximal composition, fatty acid profile, instrumental colour, texture analysis, and lipid oxidation were measured.

Materials and methods

Microalgae biomass and experimental diets

Nannochloropsis gaditana biomass (44.5% crude protein, 33.3% carbohydrates, 4.5% ash, and 17.7% crude lipid on dry matter basis) was obtained from EU-H2020 SABANA facilities of the Universidad de Almería (Spain). Enzymatic hydrolysis was carried out by mixing *N. gaditana* meal, at a final concentration of 150 g dry weight L^{-1} in 50 mM sodium citrate buffer solution (pH 5.5), and incubated at 45 ºC under continuous agitation for 5 h as described in Sáez et al. (2022).

Five iso-nitrogenous (45.5%, DW) and iso-lipidic (15.5%, DW) experimental feeds were formulated; two of them contained 25 and 50 g kg^{-1} *N. gaditana* raw biomass (labelled as R25 and R50, respectively); other two experimental groups included 25 and 50 g kg^{-1} *N. gaditana* hydrolysates (designated as H25 and H50, respectively), and a fifth diet, microalgae-free, was used as the control batch (CT). The proximal composition and fatty acid profiles of the experimental aquafeeds are shown in Tables 1 and 2, respectively.

Diets were formulated and manufactured at the CEIA$_3$-Universidad de Almería facilities (Servicio de Piensos Experimentales, http://www.ual.es/stecnicos_spe) (Almeria, Spain) using standard aquafeed extrusion processing procedures.

Fish maintenance and experimental design

The feeding trial was carried out at the aquaculture facilities (REGA: ES300261040017) of Centro Oceanográfico

Table 1 Ingredient composition of the experimental diets

Ingredient composition (% dry matter)	Diets				
	CT	R25	R50	H25	H50
Fish meal LT94[1]	15.0	15.0	15.0	15.0	15.0
Raw *N. gaditana*[2]	–	2.5	5.0	–	–
Hydrolysed *N. gaditana*	–	–	–	2.5	5.0
Squid meal[3]	2.0	2.0	2.0	2.0	2.0
CPSP90[4]	1.0	1.0	1.0	1.0	1.0
Krill meal[5]	2.0	2.0	2.0	2.0	2.0
Gluten meal[6]	15.0	15.0	15.0	15.0	15.0
Soybean protein concentrate[7]	40.0	38.8	37.3	38.8	37.3
Fish oil[8]	11.4	11.0	10.5	11.0	10.5
Soybean lecithin[9]	1.0	1.0	1.0	1.0	1.0
Wheat meal[10]	5.4	4.5	4.0	4.5	4.0
Choline chloride[11]	0.5	0.5	0.5	0.5	0.5
Betain[12]	0.5	0.5	0.5	0.5	0.5
Lysine[13]	1.5	1.5	1.5	1.5	1.5
Methionine[14]	0.6	0.6	0.6	0.6	0.6
Vitamin and mineral premix[15]	2.0	2.0	2.0	2.0	2.0
Vitamin C[16]	0.1	0.1	0.1	0.1	0.1
Guar gum[17]	2.0	2.0	2.0	2.0	2.0
Crude protein	45.2 ± 0.2	46.1 ± 1.1	46.4 ± 0.1	45.4 ± 0.5	45.9 ± 1.0
Crude lipid	15.8 ± 0.1	15.1 ± 0.2	15.5 ± 0.1	15.4 ± 0.2	15.1 ± 0.1
Ash	7.1 ± 0.2	7.9 ± 0.1	8.3 ± 0.1	7.6 ± 0.6	8.5 ± 0.1
Moisture	6.1 ± 0.2	5.8 ± 0.2	5.3 ± 0.1	5.9 ± 0.5	4.9 ± 0.1

CT: control diet. R25 and R50: diets including 25 and 50 g kg^{-1} raw microalgal biomass, respectively. H25 and H50: diets including 25 and 50 g kg^{-1} hydrolysed microalgae, respectively

[1]69.4% crude protein, 12.3% crude lipid (Norsildemel, Bergen, Norway);

[2]*Nannochloropsis gaditana* (44.5% crude protein, 33.3% carbohydrates, 4.5% ash, and 17.7% crude lipid);

[3,4,5] purchased from Bacarel (UK). CPSP90 is enzymatically pre-digested fishmeal

[6]78% crude protein (Lorca Nutrición Animal SA, Murcia, Spain)

[7]Soybean protein hydrolysate, 65% crude protein, 8% crude lipid (DSM, France)

[8]AF117DHA (Afamsa, Spain)

[9]P700IP (Lecico, DE)

[10]Local provider (Almería, Spain)

[11,12,13,14]Lorca Nutrición Animal SA (Murcia, Spain)

[15]Lifebioencapsulation SL (Almería, Spain). Vitamins (mg kg^{-1}): vitamin A (retinyl acetate), 2,000,000 UI; vitamin D3 (DL-cholecalciferol), 200,000 UI; vitamin E (Lutavit E50), 10,000 mg; vitamin K3 (menadione sodium bisulphite), 2,500 mg; vitamin B1(thiamine hydrochloride), 3,000 mg; vitamin B2 (riboflavin), 3,000 mg; calcium pantothenate, 10,000 mg; nicotinic acid, 20,000 mg; vitamin B6 (pyridoxine hydrochloride), 2,000 mg; vitamin B9 (folic acid), 1,500 mg; vitamin B12 (cyanocobalamin), 10 mg vitamin H (biotin), 300 mg; inositol, 50,000 mg; betaine (Betafin S1), 50,000 mg. Minerals (mg kg^{-1}): Co (cobalt carbonate), 65 mg; Cu (cupric sulphate), 900 mg; Fe (iron sulphate), 600 mg; I (potassium iodide), 50 mg; Mn (manganese oxide), 960 mg; Se (sodium selenite), 1 mg; Zn (zinc sulphate) 750 mg; Ca (calcium carbonate), 18.6%; (186,000 mg); KCl, 2.41%; (24,100 mg); NaCl, 4.0% (40,000 mg)

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de Murcia (Mazarrón, Spain), Instituto Español de Oceanografía -CSIC. 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. The experimental procedures were approved by in accordance with IEO-CSIC Ethics Committee for Animal Experimentation, and the approval of the Ministry of Water, Agriculture and Environment of the Autonomous Community Region of Murcia (Spain; #A13200101).

Table 2 Fatty acid profile of *Nannochloropsis gaditana* meal and the experimental diets (% of total fatty acids)

Fatty acids	*N. gaditana*	Diets					*P*
		CT	R25	R50	H25	H50	
14:00	5.60 ± 0.01	2.76 ± 0.01^{b}	2.81 ± 0.02^{c}	2.82 ± 0.01^{c}	2.74 ± 0.01	2.73 ± 0.01^{a}	*0.003*
16:00	22.4 ± 0.02	19.83 ± 0.06^{a}	20.24 ± 0.08^{b}	20.33 ± 0.06^{b}	19.85 ± 0.04^{a}	19.96 ± 0.08^{a}	*0.002*
18:00	21.30 ± 0.02	5.53 ± 0.00^{e}	5.44 ± 0.01^{d}	5.39 ± 0.01^{c}	5.35 ± 0.03^{b}	5.29 ± 0.02^{a}	*< 0.001*
16:1n7	21.30 ± 0.02	3.95 ± 0.04^{a}	4.44 ± 0.03b	4.80 ± 0.01^{e}	4.22 ± 0.01^{b}	4.71 ± 0.01^{d}	*< 0.001*
18:1n7		1.92 ± 0.01	1.93 ± 0.01	1.90 ± 0.01	1.89 ± 0.05	1.91 ± 0.02	*n.s*
18:1n9		14.13 ± 0.08^{c}	13.98 ± 0.09^{c}	13.57 ± 0.02^{a}	13.78 ± 0.03^{b}	13.59 ± 0.06^{a}	*< 0.001*
20:1n9	4.0 ± 0.01	1.66 ± 0.02	1.63 ± 0.02	1.69 ± 0.12	1.61 ± 0.01	1.60 ± 0.00	*n.s*
18:2n6		11.15 ± 0.07^{ab}	11.31 ± 0.08^{ab}	11.12 ± 0.00^{ab}	11.40 ± 0.01^{b}	11.07 ± 0.12^{a}	*0.0233*
18:3n3	3.7 ± 0.01	1.18 ± 0.01^{b}	1.18 ± 0.01^{b}	1.15 ± 0.00^{a}	1.18 ± 0.01^{b}	1.15 ± 0.01^{a}	*n.s*
16:2n4		0.85 ± 0.01	0.86 ± 0.00	0.87 ± 0.01	0.84 ± 0.01	0.86 ± 0.01	*n.s*
16:3n4		0.95 ± 0.00^{b}	0.94 ± 0.01^{ab}	0.93 ± 0.01^{ab}	0.92 ± 0.00^{a}	0.91 ± 0.00^{a}	*0.001*
18:4n3		0.57 ± 0.01	0.56 ± 0.02	0.57 ± 0.04	0.59 ± 0.02	0.58 ± 0.03	*n.s*
20:4n6		0.31 ± 0.01^{ab}	0.26 ± 0.01^{a}	0.29 ± 0.01^{ab}	0.38 ± 0.04^{b}	0.36 ± 0.04^{b}	*0.019*
20:4n3	9.5 ± 0.02	1.83 ± 0.01^{b}	1.34 ± 0.00^{a}	1.33 ± 0.01^{a}	2.21 ± 0.10^{c}	1.94 ± 0.04^{b}	*< 0.001*
20:5n3 (EPA)	33.4 ± 0.05	5.95 ± 0.00^{a}	6.51 ± 0.07^{b}	6.94 ± 0.00^{b}	6.28 ± 0.02^{b}	6.82 ± 0.03^{b}	*< 0.001*
22:5n3		1.46 ± 0.02	1.43 ± 0.03	1.40 ± 0.01	1.42 ± 0.01	1.41 ± 0.02	*n.s*
22:6n3 (DHA)		17.42 ± 0.12^{d}	16.85 ± 0.09^{c}	16.20 ± 0.00^{a}	16.58 ± 0.09^{b}	16.35 ± 0.01^{a}	*< 0.001*
Others		13.43 ± 0.12^{b}	8.27 ± 0.49^{a}	8.69 ± 0.04^{a}	8.77 ± 0.20^{a}	8.78 ± 0.14^{a}	*< 0.001*
∑SFA		28.12 ± 0.07^{a}	28.50 ± 0.11^{b}	28.55 ± 0.08^{b}	27.94 ± 0.07^{a}	27.98 ± 0.11^{a}	*0.003*
∑MUFA		21.67 ± 0.15^{ab}	21.98 ± 0.07^{b}	21.96 ± 0.11^{b}	21.50 ± 0.08^{a}	21.80 ± 0.05^{ab}	*0.021*
∑PUFA		39.88 ± 0.22^{b}	39.45 ± 0.30^{ab}	38.99 ± 0.05^{a}	40.04 ± 0.03^{b}	39.68 ± 0.04^{b}	*0.001*
∑n-3		28.42 ± 0.17^{b}	27.88 ± 0.21^{a}	27.59 ± 0.06^{a}	28.26 ± 0.01^{b}	28.25 ± 0.12^{b}	*0.008*
∑n-6		11.46 ± 0.05^{ab}	11.57 ± 0.09^{b}	11.41 ± 0.01^{a}	11.78 ± 0.04^{c}	11.43 ± 0.08^{a}	*0.009*
n3/n6		2.48 ± 0.00^{b}	2.41 ± 0.00^{a}	2.42 ± 0.01^{a}	2.40 ± 0.01^{a}	2.47 ± 0.03^{b}	*0.005*
EPA/DHA		0.34 ± 0.00^{a}	0.39 ± 0.00^{b}	0.43 ± 0.00^{b}	0.38 ± 0.00^{b}	0.42 ± 0.00^{b}	*< 0.001*

Dietary treatments: CT: control diet. R25 and R50: diets including 25 and 50 g kg^{-1} raw microalgal biomass, respectively. H25 and H50: diets including 25 and 50 g kg^{-1} hydrolysed microalgae, respectively. Values with different lowercase superscript indicate significant differences in muscle lipids attributed to dietary treatments ($P < 0.05$). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid. Values are expressed as average ± sd ($n = 9$ fish per dietary treatment). *n.s.* not significant

Healthy adult specimens of gilthead seabream (450 ± 28 g average initial body weight; 30 ± 0.7 cm average total body lenght) were randomly distributed in 15 tanks (triplicate tanks per dietary treatment) of 2,000 L capacity (15 fish tank^{-1}).

Initial stock density was 3.4 kg m^{-3} and sea water renewal rate (37‰ salinity) was kept at 780 L h^{-1} in an open flow circuit maintaining values of ammonia and nitrites (< 0.1 mg L^{-1}) suitable for gilthead seabream culture. Animals were kept under 12L:12D photoperiod and natural temperature, thus, the water temperature increased gradually from 17 ºC at the beggining of the feeding trial to 21 ºC during the assay. Light intensity ranged from 50 to 70 lx. Tanks were equipped with aerators to maintain an adequate level of oxygenation (above 6 mg L^{-1}).

Fish were fed with a commercial diet (CT diet) during a 15-day acclimation period prior to the beggining of the feeding trial. Afterwards, the experimental diets were offered ad libitum thrice per day (9:00, 14:00 and 19:00), until a máximum of 1.2% of the tank biomass, during 42 days. The amount of feed ingested was recorded daily in each tank.

At the end of the feeding trial (42 days) ten fish per tank (30 animals per dietary treatment and sampling time) were withdrawn individually weighed and measured, and killed by anaesthetic overdose (200 mg L^{-1} clove oil; isoeugenol) followed by spine severing. The rest of the animals were kept for a different study.

Immediately after slaughtering, specimens were gutted, filleted and packed in transparent sterile polyethylene bags. These bags were directly stored at 4 ºC in a cold room (4 ºC ± 1 ºC) for a period of 15 days with the aim of assessing changes in fillet quality parameters throughout the shelf life. Samples were withdrawn from each lot at 1, 2, 4, 7, 9, 11 and 15 days *post-mortem* (dpm), and total viable counts, lipid

oxidation, pH, water holding capacity (WHC), texture profile analysis (TPA), and skin and flesh instrumental colour were determined at each sampling time in dorsal muscle of 4 fillets per treatment.

Biometric parameters

The biometric parameters measured on fish were total length, total weight, liver and gonad weight, and fillet weight. From these data, biometric indices were estimated in accordance with the following equations: i) hepatosomatic index (HSI = (100 × liver weight) /fish weight); ii) gonadosomatic index (GSI = (100 × gonad weight) /fish weight); and iii) fillet yield (FY = (100 × 2 fillet weight) /fish weight).

Proximate composition and fatty acid profile of fillets

Samples of the anterior dorsal muscle of seabream fillets were obtained by cutting into cubes (approx. 2 g) and then freeze-dried. A pool of samples from 9 fillets from each treatment was prepared and used for proximate composition and fatty acid profile analysis. Proximate analysis (dry matter, ash, and crude protein, N × 6.25) of aquafeeds and muscle samples were determined according to AOAC (2000) protocols. Lipids were extracted following Folch et al. (1957) methodology using chloroform/methanol (2:1 v/v) as solvent, and total lipid content was calculated gravimetrically. Fatty acid profiles of *N. gaditana*, diets and muscle samples were determined by gas chromatography (Hewlett Packard, 4890 Series II, Hewlett Packard, USA) following the method described in Rodríguez-Ruiz et al. (1998), using a modification of the direct transesterification method described by Lepage and Roy (1984) that requires no prior separation of the lipid fraction. Based on FA profile data, the index of atherogenicity (IA) and the index of thrombogenicity (IT) were calculated according to Ulbricht and Southgate (1991). Namely, index of atherogenicity = (12:0 + 4 * 14:0 + 16:0) / [(*n*-6 + *n*-3) PUFAs + 18:1 + other MUFAs]; index of thrombogenicity = (14:0 + 16:0 + 18:0) / [(0.5 * 18:1) + (0.5 * ΣMUFAs) + (0.5 * *n*-6 PUFAs) + (3 * *n*-3 PUFAs) + (*n*-3/*n*-6)], where MUFAs and PUFAs stand for unsaturated fatty acids and polyunsaturated fatty acids, respectively.

Total viable counts (TVC)

The determination of total viable psychrophilic bacterial counts (TVC) was carried out on fillet samples up to 15 dpm (days *post-mortem*) according to Sáez et al. (2020). Briefly, the anterior dorsal muscle of seabream was cut into cubes (1 g), transferred aseptically to sterile tubes containing 10 mL of 0.1% (w/v) peptone water (Cultimed, Spain), and homogenized for 60 s using a sterile mechanical homogenizer (Polytron PT-2100, Kinematica AG, Switzerland). TVC of psychrophilic bacterial were quantified using plate count agar (PCA, Merck), after incubation for 120 h at 4 ºC. Microbiological loads were expressed as logarithm of colony-forming units (cfu) per gram of muscle.

Lipid oxidation

Fillet lipid oxidation was assessed by thiobarbituric acid-reactive substances (TBARS) analysis according to Buege and Aust (1978). Briefly, muscle samples (1 g each) were homogenized in 4 mL 50 mM NaH_2PO_4, 0.1% (v/v) Triton X-100 solution. The mixture was centrifuged (10,000 × *g*, 20 min, 4 ºC) and supernatants were mixed in a ratio 1:5 (v/v) with 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). The mixture was heated in boiling water (100 ºC) for 15 min and then centrifuged (3,600 × *g*, 10 min, 4 ºC), and the absorbance of supernatants was measured at 535 nm. The amount of TBARS was expressed as mg of malonyl dialdehyde (MDA) per kg of muscle after comparing with a MDA standard.

pH and water holding capacity (WHC)

Flesh pH was determined in dorsal muscle by means of a penetration electrode (Crison, model GLP 21; sensitivity 0.01 pH units) as described in Suárez et al. (2010). WHC (expressed in percentage) was calculated from a piece (1 cm^3) of the anterior part of dorsal muscle as the difference between the initial percentage of water and the percentage of water released after centrifugation, as detailed in Suárez et al. (2010).

Texture profile analysis (TPA)

Fillet texture was measured on the skin side of fillets, on the dorsal muscle, by compression of and area anterior 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, controlled with Texture Expert Exceed 2.52 software (Stable Micro Systems, England). 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^{-1}. The textural parameters hardness (maximum force required to compress the sample), springiness (ability of the sample to recover its original form after removing the deforming force), cohesiveness (extent to which the sample could be deformed prior to rupture), gumminess (force needed to disintegrate a semisolid sample to a steady state of swallowing), chewiness (the work needed to

chew a solid sample to reach a steady state of swallowing) and resilience (how well a product fights to regain its original position) were calculated as described in Bourne (1978).

Instrumental colour measurement

Colour was measured thrice on dorsal side of skin, as well as on flesh fillets by L^*, a^*, and b^* system (CIE 1986), by Minolta Chroma meter CR400 device (Minolta, Japan). The brightness (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 determined.

Statistics

The effect of the categorical variables "microalgae inclusion level", "pre-treatment", and "*post-mortem* time", as well as their interactions, were determined for each numeric parameter studied by fitting a generalized lineal statistical model (GLM analysis) that relates measured parameters to predictive factors, using specific software (SPSS 22, IBM Corp. Inc.). Least squares means were tested for differences using Fisher's least significant difference (LSD) procedure. Unless otherwise is 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 acid profile), arcsine transformation of their square root was carried out in order to normalize data prior to the statistical analysis.

Results

Biometric parameters

After the 42-day feeding trial, fish biometric parameters and fillet yield were recorded (Table 3), and the results indicated that none of the indices studied showed significant differences ($P > 0.05$).

Proximate analysis and fatty acid profile of fillets

With regard to muscle composition, no significant differences in moisture and ash contents were observed among the

Table 3 Fish body biometric parameters at day 42 of the feeding trial

Parameters	Diets					*P*
	CT	R25	R50	H25	H50	
Total weight (g)	508.83 ± 18.19	497.5 ± 40.61	502.5 ± 26.58	504.17 ± 29.93	515.5 ± 27.74	*n.s*
Total length (cm)	31.08 ± 0.86	30.92 ± 1.07	30.4 ± 0.85	30.67 ± 0.88	30.83 ± 0.61	*n.s*
Hepatic weight (g)	5.4 ± 0.35	5.16 ± 0.71	5.19 ± 1.04	4.76 ± 1.1	5.81 ± 0.34	*n.s*
Gonads weight (g)	0.72 ± 0.22	1.02 ± 0.45	1.41 ± 0.61	0.95 ± 0.32	0.82 ± 0.43	*n.s*
Fillet weight (g)	153.53 ± 7.15	153.57 ± 11.02	156.7 ± 11.58	149.93 ± 13.6	150.08 ± 16.96	*n.s*
HIS (%)	1.06 ± 0.07	1.04 ± 0.15	1.03 ± 0.17	0.94 ± 0.19	1.13 ± 0.07	*n.s*
GSI (%)	0.14 ± 0.04	0.2 ± 0.08	0.28 ± 0.12	0.19 ± 0.06	0.16 ± 0.08	*n.s*
Fillet yield (%)	60.39 ± 3.13	62.13 ± 7.48	62.59 ± 6.51	59.82 ± 8.06	58.51 ± 8.5	*n.s*

Dietary treatments: CT: control diet. R25 and R50: diets including 25 and 50 g kg^{-1} raw microalgal biomass, respectively. H25 and H50: diets including 25 and 50 g kg^{-1} hydrolysed microalgae, respectively. Values with different lowercase superscript indicate significant differences attributed to dietary treatments ($P < 0.05$). Values are expressed as average ± sd ($n = 30$ fish per dietary treatment). *n.s.* not significant

Table 4 Effects of the dietary inclusion of *N. gaditana* on muscle composition of seabream fillets at 42 days of the feeding trial

Parameters	Diets					*P*
	CT	R25	R50	H25	H50	
Crude protein	23.13 ± 0.50^a	24.21 ± 0.66^b	24.56 ± 0.29^b	25.67 ± 0.33^c	25.90 ± 0.31^c	*< 0.01*
Total lipid	5.29 ± 0.07^c	4.54 ± 0.07^a	4.56 ± 0.09^a	4.61 ± 0.03^b	4.60 ± 0.06^b	*< 0.01*
Ash	1.69 ± 0.06	1.70 ± 0.08	1.75 ± 0.04	1.70 ± 0.06	1.65 ± 0.06	*n.s*
Moisture	73.18 ± 0.55	73.54 ± 0.62	73.47 ± 0.34	72.98 ± 0.45	72.31 ± 0.39	*n.s*

Dietary treatments: CT: control diet. R25 and R50: diets including 25 and 50 g kg^{-1} raw microalgal biomass, respectively. H25 and H50: diets including 25 and 50 g kg^{-1} hydrolysed microalgae, respectively. Values with different lowercase superscript indicate significant differences attributed to dietary treatments ($P < 0.05$). Values are expressed as average ± sd ($n = 9$ fish per dietary treatment). *n.s.* not significant

experimental groups (Table 4). Nevertheless, dietary treatments including *N. gaditana* yielded lower total lipid and higher crude protein contents in fillets compared to controls, irrespectively of the inclusion level.

Muscle fatty acid (FA) composition of gilthead seabream fillets is summarized in Table 5. PUFAs (from 24 to 30% of FAs) and monounsaturated fatty acids (MUFA, 26–28%) were the prevailing FAs in fish muscle at the end of the feeding trial whatever the dietary treatment considered, followed by saturated fatty acids (SFAs, 19–21%). Overall, the effect of microalgae inclusion in diets on muscle FA profile was scarce, although significant increase in total n-3 PUFA (owing to higher EPA and DHA) content was observed compared to control fish batch. Inclusion level yielded significant effects on ∑PUFA ($P = 0.036$) and ∑n-3 PUFA ($P < 0.01$). On the other hand, for a given inclusion level, the microalgae enzyme pretreatment only increased 20:4n-3 content in muscle ($P = 0.035$).

Considered individually, oleic acid (18:1n9) was the predominant fatty acid (19–21%) in all batches, followed by docosahexaenoic acid (DHA, 22:6n-3; 12–16%) and palmitic acid (16:0; 13–14%). Differences among experimental groups were observed, with higher proportion of SFAs and MUFAs in control specimens, while in *N. gaditana*-fed fish the proportion of n-3 PUFA was significantly higher. A dose-dependent effect was observed, mainly in n-3 PUFA

Table 5 Effects of the dietary inclusion of *N. gaditana* on fatty acid (FA) profile of gilthead seabream (*S. aurata*) muscle after a 42-day feeding trial (% of total FAs)

FA	Diets					*P*
	CT	R25	R50	H25	H50	
14:00	1.53 ± 0.31	1.28 ± 0.12	1.49 ± 0.36	1.34 ± 0.08	1.34 ± 0.05	*n.s*
16:00	14.72 ± 0.49^{b}	13.37 ± 0.27^{a}	13.89 ± 0.58^{a}	13.91 ± 0.34^{a}	13.50 ± 0.19^{a}	*0.007*
16:1n7	2.69 ± 0.12^{b}	2.40 ± 0.09^{a}	2.48 ± 0.1^{a}	2.46 ± 0.03^{a}	2.46 ± 0.04^{a}	*0.030*
16:2n4	0.29 ± 0.04^{b}	0.25 ± 0.01^{a}	0.24 ± 0.02^{a}	0.25 ± 0.00^{a}	0.27 ± 0.02^{a}	*0.022*
16:3n4	0.31 ± 0.01^{b}	0.29 ± 0.03^{a}	0.31 ± 0.00^{a}	0.29 ± 0.00^{a}	0.28 ± 0.01^{a}	*0.003*
18:00	5.68 ± 0.10^{b}	5.02 ± 0.11^{ba}	5.10 ± 0.03^{a}	5.19 ± 0.36^{a}	5.15 ± 0.08^{a}	*0.001*
18:1n9	21.41 ± 0.03^{c}	20.31 ± 0.07^{b}	20.22 ± 0.23^{b}	19.88 ± 0.24^{a}	19.91 ± 0.66^{a}	*< 0.001*
18:1n7	1.71 ± 0.06	1.68 ± 0.05	1.70 ± 0.09	1.72 ± 0.08	1.73 ± 0.08	*n.s*
18:2n6	12.31 ± 0.35	12.41 ± 0.15	12.05 ± 0.18	12.76 ± 0.21	12.43 ± 0.35	*n.s*
18:3n3	2.28 ± 0.06	2.24 ± 0.16	2.25 ± 0.08	2.24 ± 0.13	2.16 ± 0.20	*n.s*
18:4n3	0.47 ± 0.02	0.46 ± 0.02	0.46 ± 0.05	0.46 ± 0.02	0.44 ± 0.04	*n.s*
20:1n9	2.77 ± 0.08	2.66 ± 0.04	2.71 ± 0.28	2.72 ± 0.03	2.76 ± 0.27	*n.s*
20:4n6	1.33 ± 0.12	1.41 ± 0.06	1.35 ± 0.04	1.33 ± 0.06	1.37 ± 0.03	*n.s*
20:4n3	0.87 ± 0.14^{a}	1.06 ± 0.09^{b}	0.99 ± 0.00^{ab}	1.17 ± 0.11^{c}	1.23 ± 0.05^{c}	*0.006*
20:5n3	4.97 ± 0.05^{a}	5.42 ± 0.15^{b}	6.23 ± 0.08^{c}	5.45 ± 0.13^{b}	6.37 ± 0.19^{c}	*< 0.001*
22:5n3	2.38 ± 0.13^{a}	2.56 ± 0.10^{b}	2.64 ± 0.07^{bc}	2.71 ± 0.08^{bc}	2.73 ± 0.00^{c}	*0.004*
22:6n3	12.05 ± 0.05^{a}	15.08 ± 0.3^{b}	15.31 ± 0.13^{c}	15.11 ± 0.25^{b}	15.93 ± 0.59^{d}	*< 0.001*
Others	12.57 ± 2.12	12.11 ± 0.21	10.58 ± 0.41	11.01 ± 0.61	9.93 ± 0.42	
∑SFA	21.93 ± 0.82^{b}	19.67 ± 0.10^{a}	20.48 ± 0.41^{ab}	20.68 ± 0.45^{ab}	19.99 ± 0.45^{a}	*0.001*
∑MUFA	28.24 ± 1.13^{b}	27.05 ± 0.06^{a}	27.51 ± 0.29^{a}	26.47 ± 0.47^{a}	26.20 ± 0.68^{a}	*< 0.001*
∑PUFA	24.35 ± 0.15^{a}	28.22 ± 0.46^{b}	29.23 ± 0.05^{bc}	28.48 ± 0.66^{b}	30.23 ± 0.45^{c}	*< 0.001*
∑n-3	23.02 ± 0.10^{a}	26.81 ± 0.49^{b}	27.885 ± 0.30^{c}	27.14 ± 0.42^{bc}	28.86 ± 0.47^{d}	*< 0.001*
∑n-6	13.64 ± 0.32	13.82 ± 0.15	13.4 ± 0.20	14.09 ± 0.15	13.80 ± 0.38	*n.s*
∑n-9	23.84 ± 1.20^{b}	22.97 ± 0.11^{a}	22.93 ± 0.16^{a}	22.60 ± 0.27^{a}	22.67 ± 0.92^{a}	*0.001*
∑n-3 HUFA	20.27 ± 0.12^{a}	24.12 ± 0.37^{b}	25.17 ± 0.01^{c}	24.44 ± 0.50^{b}	26.25 ± 0.50^{d}	*< 0.001*
n3/n6	1.69 ± 0.03^{a}	1.94 ± 0.06^{b}	2.08 ± 0.05^{c}	1.93 ± 0.04^{b}	2.09 ± 0.07^{c}	*< 0.001*
EPA/DHA	0.41 ± 0.00^{c}	0.36 ± 0.01^{a}	0.41 ± 0.01^{c}	0.36 ± 0.01^{a}	0.40 ± 0.02^{b}	*< 0.001*
AI	0.32 ± 0.02^{c}	0.27 ± 0.01^{a}	0.29 ± 0.02^{b}	0.28 ± 0.01^{b}	0.28 ± 0.01^{b}	*0.037*
TI	0.24 ± 0.01^{c}	0.19 ± 0.01^{a}	0.19 ± 0.00^{a}	0.20 ± 0.00^{b}	0.19 ± 0.00^{a}	*< 0.001*

Dietary treatments: CT: control diet. R25 and R50: diets including 25 and 50 g kg^{-1} raw microalgal biomass, respectively. H25 and H50: diets including 25 and 50 g kg^{-1} hydrolysed microalgae, respectively. Values with different lowercase superscript indicate significant differences in muscle lipids attributed to dietary treatments ($P < 0.05$). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid. AI and TI: atherogenic and thrombogenic indices, respectively, as explained in M&M section. Values are expressed as average ± sd ($n = 9$ fish per dietary treatment). *n.s.* not significant

content, whereas the influence of the enzyme pre-treatment on FA profile was scarce. Overall, both atherogenic and trombogenic indices were reduced signicantly as a result of microalgae inclusion in diets.

Total viable counts (TVC)

Psychrophilic bacterial counts in fillets subjected to the different experimental treatments are shown in Fig. 1. Overall, *N. gaditana* supplement ($P<0.01$) and storage time ($P<0.01$) were responsible for significant differences in this parameter. Predictably, bacterial counts increased throughout storage time, but microalgae inclusion reduced microbial growth up to 15 days post-mortem (dpm) compared to control batch. However, differences were significant only from 7 dpm onwards. No significant effects on TVC values attributable to the variables inclusion level ($P=0.581$) or biomass enzymatic pre-treatment ($P=0.989$) were observed.

Muscle lipid oxidation

Lipid oxidation in fillets clearly depended on storage time in all the experimental groups ($P<0.01$), as evidenced by the significant increase of this parameter from the beginning to the end of the cold storage period (Fig. 2). As a whole, no significant effects attributable to microalgae dosage ($P=0.167$) or pre-treatment ($P=0.475$) were observed at initial stages (1 and 2 dpm).

From 4 dpm onwards, CT fillets yielded higher TBARS values compared with any of the batches fed with *N. gaditana* ($P<0.01$). Although fillets from fish fed with 5% (R50 and H50) *N. gaditana* tended to show lower lipid oxidation than 2.5% (R25 and H25) however, differences were significant only at later stages of the storage period (after 11 dpm). With regard to the influence of the microalgae pre-treatment, TBARS contents tended to be lower throughout the complete storage period in hydrolysed batches, although statistical difference was observed only in the last sampling point (15 dpm) between R50 and H50 experimental groups.

pH and water holding capacity (WHC)

With regard to pH (Fig. 3A), overall, both inclusion level and storage time led to significant differences ($P<0.01$). Values of pH increased throughout storage time in all the experimental groups. The most evident effect observed

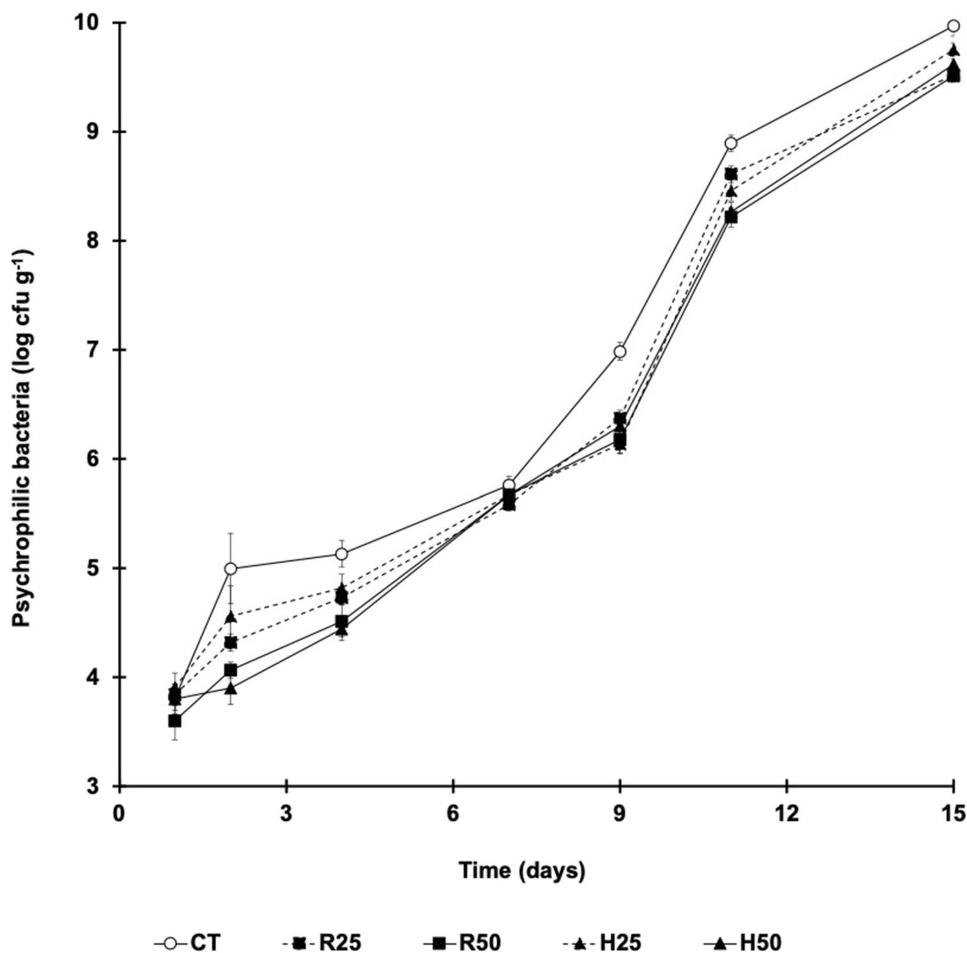

Fig. 1 Time-course of changes in total viable counts (TVC, log CFU g^{-1}) in seabream fillets during a 15-day cold storage (4 ºC) period. Experimental groups were: CT: control diet. R25 and R50: diets including 25 and 50 g kg^{-1} raw microalgal biomass, respectively. H25 and H50: diets including 25 and 50 g $kg.^{-1}$ hydrolysed microalgae, respectively. Values are expressed as average ± sd (n = 4 fillets per dietary treatment and sampling time)

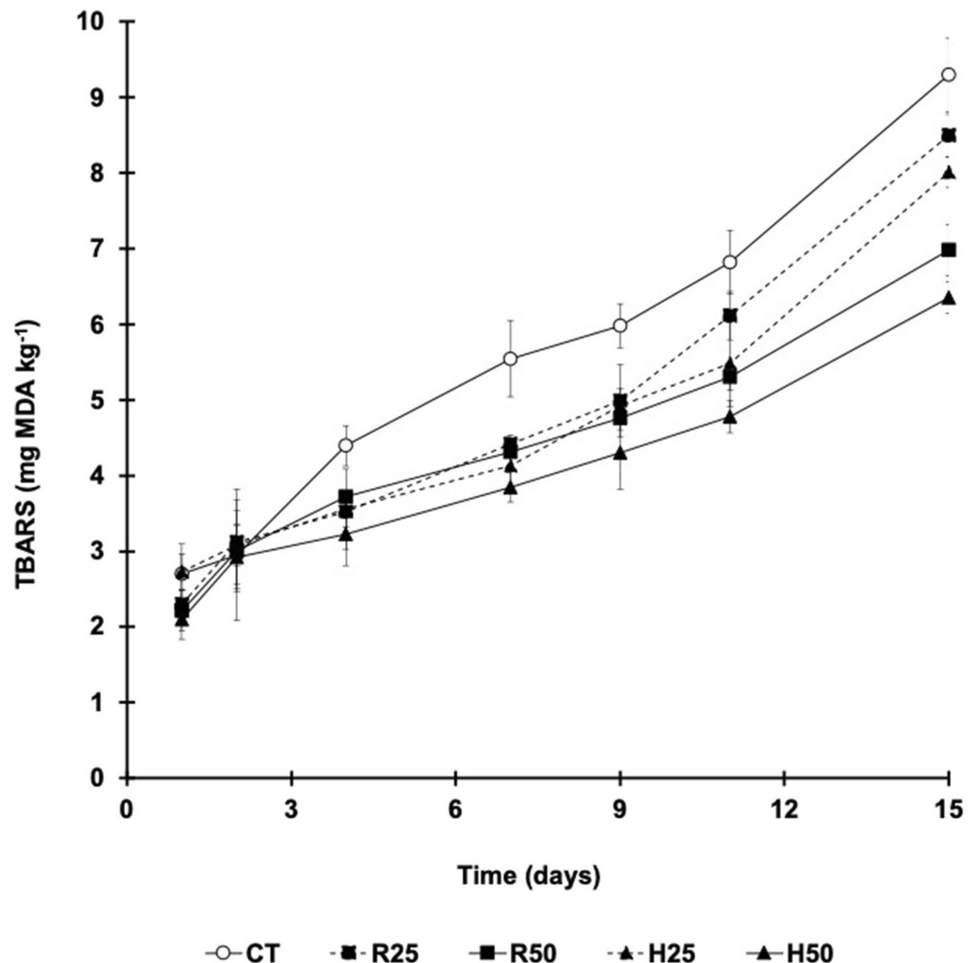

Fig. 2 Lipid oxidation (estimated by TBARS content) of seabream fillets during a 15-day cold storage (4 ºC) period. Experimental groups were: CT: control diet. R25 and R50: diets including 25 and 50 g kg^{-1} raw microalgal biomass, respectively. H25 and H50: diets including 25 and 50 g kg^{-1} hydrolysed microalgae, respectively. Values are expressed as average ± sd (n = 4 fillets per dietary treatment and sampling time)

was that all the fillets from fish fed with microalgae-containing diets yielded consistently lower pH values than controls ($P < 0.01$). On the other hand, with some exceptions (9 dpm), the higher the microalgae inclusion, the lower the pH values, whereas no influence of the biomass pre-treatment was observed throughout the storage period ($P = 0.069$).

With regard to WHC (Fig. 3B), this parameter decreased significantly throughout the storage time in all the experimental groups. Compared to CT fillets, the inclusion of the microalgal biomass in diets did not cause significant effects on WHC ($P = 0.055$). Considered separately, the variable "pre-treatment" ($P = 0.353$) did not yield clear effects on the results. However, the variable "inclusion level" caused significant differences in WHC, especially when raw biomass was considered (R50 vs. R25; $P < 0.001$).

Texture profile analysis (TPA)

The effects of the experimental diets on fillet textural parameters measured in dorsal muscle are summarized in Fig. 4 and Table 6. With regard to hardness (Fig. 4), considering data as a whole, the inclusion of *N. gaditana* in diets yielded higher values ($P < 0.01$) for this parameter compared to control fillets throughout the complete cold-storage period. Hardness was clearly higher in all microalgae-fed experimental batches compared to CT at the beginning of the storage period (1 dpm). The 5% dosage kept marked differences ($P < 0.01$) with CT throughout the complete storage period.

Storage time decreased markedly hardness in all experimental lots, whereas roughly, muscle softness was delayed in microalgae-fed fish, with the exception of H25 batch up to 9 dpm.

Roughly, no clear tendency could be observed for the parameters springiness, cohesiveness, gumminess, and chewiness (Table 6) owing to the dietary treatments, even if some statistical differences were observed for some sampling points. With regard to the parameter resilience, values decreased throughout the storage period for all experimental groups. Nevertheless, no differences could be attributed to the dietary factors considered ($P = 0.087$).

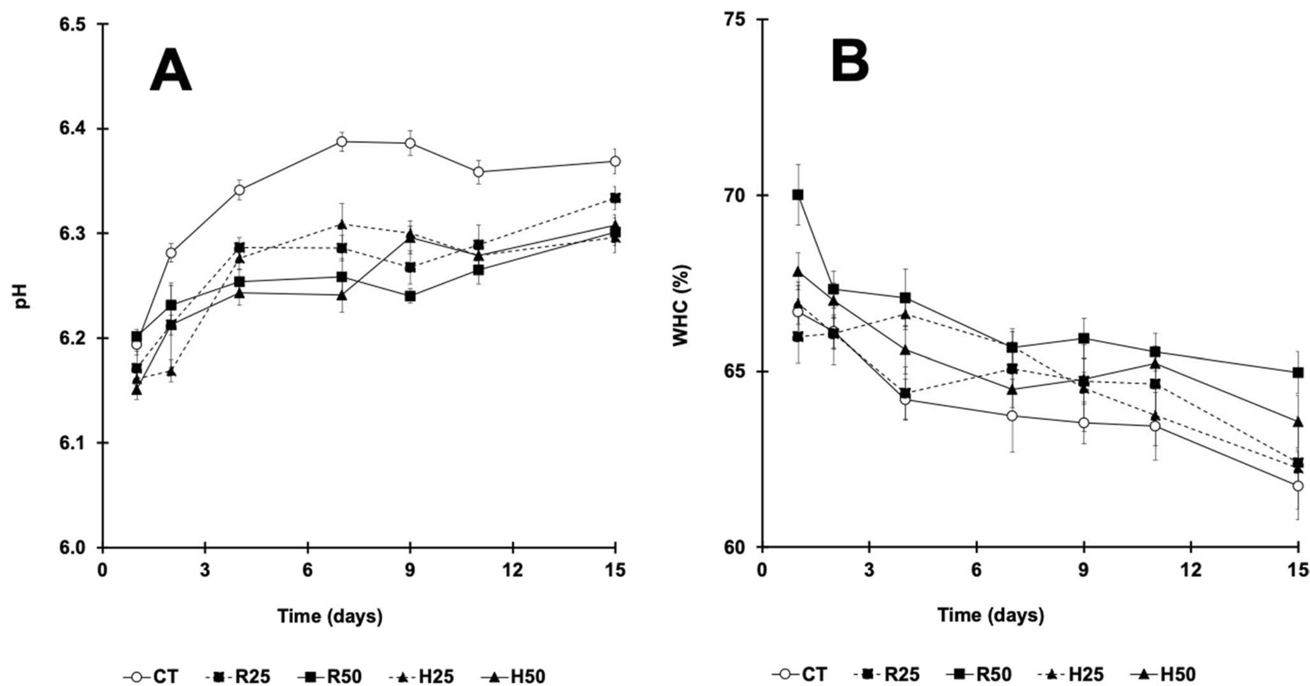

Fig. 3 Changes in seabream fillets pH and water holding capacity (WHC) during a 15-day cold storage (4 ºC) period. **A**: pH and **B**: WHC. Experimental groups were: CT: control diet. R25 and R50: diets including 25 and 50 g kg^{-1} raw microalgal biomass, respectively. H25 and H50: diets including 25 and 50 g kg^{-1} hydrolysed microalgae, respectively. Values are expressed as average ± sd (n = 4 fillets per dietary treatment and sampling time)

Instrumental colour measurement

Instrumental colour parameters are shown in Tables 7 (skin) and 8 (muscle). Skin colour was influenced by dietary *N. gaditana* inclusion, storage time and their interaction ($P \leq 0.05$). Also, significant effects attributable to both microalgae inclusion level and biomass pre-treatment were observed for some parameters.

Roughly, at the beginning of the storage period, skin of microalgae-fed lots showed higher values for L^* (more lightness), b^* (more yellowish), but lower values for a^* (more greennish) than control fillets. Throughout the 15-day cold storage period, L^* decreased significantly in microalgae-fed lots, this parameter ending up in final values similar to those measured in controls. Values of parameter a* also decreased in all batches, although microalgae-fed experimental groups kept the greenness (negative a^* values) compared to CT up to 11 dpm. Finally, b^* parameter also decreased owing to storage time in all experimental groups, being values for this parameter in general higher in R50 and H50 fillets compared to CT lot up to 9 dpm.

Colour parameters measured on the flesh side of fillets (Table 8) considered overall were influenced by *N. gaditana* inclusion in diets, storage time, and their interaction ($P < 0.01$). Microalgae supplementation yielded higher values for b^* parameter in control fish ($P < 0.001$). However, no clear trend could be observed owing to the factors "inclusion level" or "biomass pre-treatment". Finally, cold storage time increased both L^* and b^* in all experimental treatments.

Discussion

The inclusion of *N. gaditana* in finishing diets up to 5% inclusion level has influenced different quality parameters of gilthead seabream fillets, such as muscle proximal composition, antimicrobial and antioxidant effects, textural parameters, and colour attributes, altogether indicating the presence of bioactive compounds in the algal biomass capable of influencing fish physiology and organoleptic attributes in this species, as previous studies also suggested for early stages of this species (Sales et al. 2021; Sáez et al. 2022).

The effectiveness of the inclusion of *N. gaditana* in the diet depends on the ability of the fish to obtain the nutrients and bioactive compounds contained in the microalgae inner cell compartment, this is, on their digestibility. Thus, it was hypothesized that a process of enzymatic hydrolysis of the microalgae cell wall could enhance the release of intracellular components. Indeed, recent studies have reported increased release of reducing sugars, free amino acids, soluble protein and polyphenols when *N. gaditana* biomass was

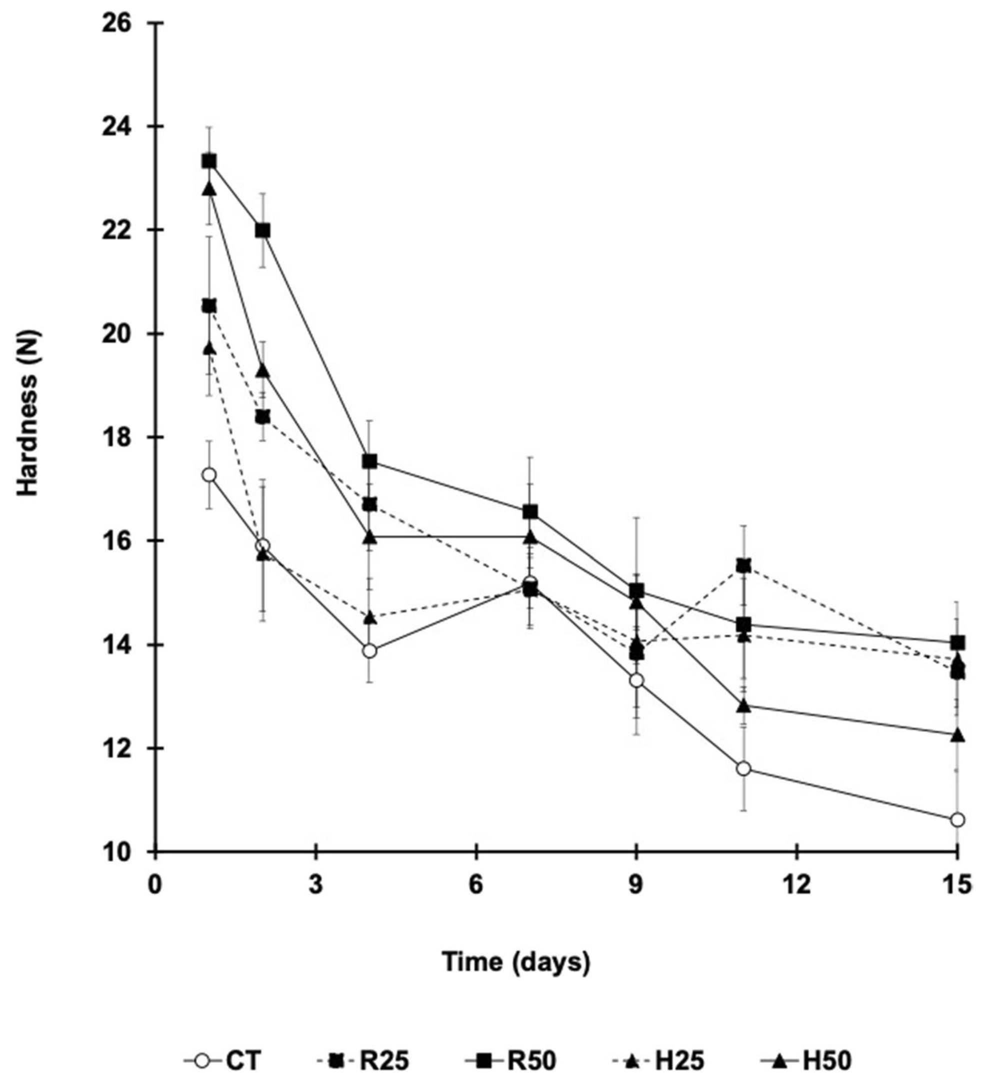

Fig. 4 Time-course of changes in seabream fillets hardness during cold storage (4 ºC). Experimental groups were: CT: control diet. R25 and R50: diets including 25 and 50 g kg^{-1} raw microalgal biomass, respectively. H25 and H50: diets including 25 and 50 g kg^{-1} hydrolysed microalgae, respectively. Values are expressed as average ± sd (n = 4 fillets per dietary treatment and sampling time)

pre-treated with cellulases (Sáez et al. 2022). Our results have shown that the microalgae hydrolysis improved some of the parameters evaluated, compared to raw biomass (such as lower muscle lipid oxidation), but this was not a general tendency, since the effects observed as a result of the enzyme hydrolysis were less evident than expected, as will be detailed below.

The lack of differences in fish growth among the experimental groups (Table 3) might be attributed to the low inclusion level considered, although it should also be taken into consideration that fish growth at this stage is slower than in earlier phases (i.e., juvenile fish), and that all the experimental diets fulfiled by far the nutritional requirements of gilthead seabream (Table 1). However, it was remarkable that muscle lipid content of fish fed with *N. gaditana*-enriched feeds was significantly lower that controls, irrespectively of dosage or biomass pre-treatment (Table 4). These results are in agreement with previous studies on gilthead sea bream fed with microalgae (Ribeiro et al. 2017; Galafat et al. 2020), as well as on other fish species (Kiron et al. 2012; Jafari et al. 2014; Khanzadeh et al. 2016; Teimouri et al. 2016). However, one of our previous studies found that *N. gaditana* at low inclusion level did not change muscle lipid content in seabream juveniles (Sáez et al. 2022). It is likely that the different stages of the life cycle considered might well have accounted for such discrepancy.

The intrinsic mechanism involved in the muscle lipid decrease owing to dietary microalgae is not yet fully understood, but it has been suggested that algae can activate fish lipid metabolism (Nematipour et al. 1990), increasing lipolysis and lipid utilization (Nakagawa et al. 2000). Nevertheless, the opposite has also been described, this is, increased muscle lipid content in both freshwater (Simanjuntak and Indarmawan Wibowo 2018; Mosha et al. 2020) and marine (He et al. 2018) fish fed with different macro and microalgae. Again, the influence of algal compounds in these phenomena remains virtually unexplained.

Table 6 Changes in texture profile analysis (TPA) parameters in seabream fillets during a 15-day cold storage (4 °C) period

Parameters	*Dpm*	Diets					*P*
		CT	R25	R50	H25	H50	
Springiness (mm)	1	0.84 ± 0.01^{D}	0.87 ± 0.01^{D}	0.86 ± 0.01^{D}	0.84 ± 0.01^{D}	0.83 ± 0.01^{C}	*n.s*
	2	0.78 ± 0.01^{C}	0.79 ± 0.03^{C}	0.82 ± 0.02^{C}	0.77 ± 0.01^{C}	0.80 ± 0.02^{C}	*n.s*
	4	$0.75\pm0.01^{b,B}$	$0.72\pm0.01^{a,B}$	$0.80\pm0.01^{d,C}$	$0.74\pm0.01^{b,B}$	$0.78\pm0.01^{c,BC}$	*≤0.001*
	7	0.73 ± 0.01^{AB}	0.74 ± 0.02^{BC}	0.76 ± 0.01^{C}	0.74 ± 0.01^{B}	0.75 ± 0.02^{BC}	*n.s*
	9	0.72 ± 0.01^{AB}	0.72 ± 0.01^{B}	0.73 ± 0.02^{AB}	0.72 ± 0.01^{AB}	0.72 ± 0.01^{B}	*n.s*
	11	0.71 ± 0.02^{A}	0.69 ± 0.01^{A}	0.71 ± 0.02^{A}	0.69 ± 0.02^{A}	0.72 ± 0.01^{B}	*n.s*
	15	0.69 ± 0.01^{A}	0.69 ± 0.01^{A}	0.68 ± 0.02^{A}	0.68 ± 0.02^{A}	0.67 ± 0.01^{A}	*n.s*
	P	*≤0.001*	*≤0.001*	*≤0.001*	*≤0.001*	*≤0.001*	
Cohesiveness	1	0.50 ± 0.01^{a}	0.52 ± 0.01^{a}	$0.57\pm0.01^{b,C}$	0.53 ± 0.01^{a}	$0.59\pm0.01^{b,D}$	*≤0.001*
	2	0.52 ± 0.01	0.51 ± 0.01	0.53 ± 0.02^{B}	0.51 ± 0.01	0.54 ± 0.01^{C}	*n.s*
	4	0.52 ± 0.01	0.50 ± 0.01	0.51 ± 0.01^{AB}	0.50 ± 0.01	0.54 ± 0.01^{BC}	*n.s*
	7	0.51 ± 0.01	0.49 ± 0.01	0.53 ± 0.01^{B}	0.50 ± 0.01	0.52 ± 0.01^{BC}	*n.s*
	9	0.51 ± 0.00	0.52 ± 0.01	0.52 ± 0.01^{B}	0.52 ± 0.02	0.53 ± 0.01^{AB}	*n.s*
	11	0.52 ± 0.02	0.52 ± 0.01	0.50 ± 0.01^{AB}	0.52 ± 0.01	0.51 ± 0.01^{AB}	*n.s*
	15	0.53 ± 0.03	0.50 ± 0.02	0.49 ± 0.01^{A}	0.54 ± 0.01	0.50 ± 0.01^{A}	*n.s*
	P	*n.s*	*n.s*	*≤0.001*	*n.s*	*≤0.001*	
Gumminess ($N\ mm^{-2}$)	1	$7.81\pm0.41^{a,C}$	$11.13\pm0.66^{b,D}$	$13.62\pm0.62^{c.E}$	$10.47\pm0.38^{b,B}$	$13.51\pm0.48^{c,D}$	*≤0.001*
	2	$8.22\pm0.62^{a,D}$	$9.27\pm0.41^{ab,C}$	$11.61\pm0.45^{c,D}$	$8.14\pm0.77^{a,A}$	$10.53\pm0.40^{bc,C}$	*≤0.001*
	4	$7.15\pm0.28^{a,BC}$	$7.90\pm0.41^{ab,AB}$	$9.07\pm0.59^{b.C}$	$7.24\pm0.43^{a,A}$	$8.57\pm0.63^{ab,B}$	*0.034*
	7	7.78 ± 0.37^{C}	7.38 ± 0.35^{AB}	8.66 ± 0.52^{BC}	7.57 ± 0.52^{A}	8.33 ± 0.48^{AB}	*n.s*
	9	6.52 ± 0.52^{BC}	7.25 ± 0.64^{AB}	7.78 ± 0.62^{AB}	7.11 ± 0.65^{A}	7.93 ± 0.35^{AB}	*n.s*
	11	$6.06\pm0.45^{a,AB}$	$8.15\pm0.49^{c,BC}$	$7.19\pm0.52^{b.AB}$	$7.44\pm0.64^{bc,A}$	$6.55\pm0.31^{ab,A}$	*0.045*
	15	$5.56\pm0.47^{a,A}$	$6.76\pm0.38^{abc,A}$	$6.83\pm0.26^{bc.A}$	$7.44\pm0.58^{c,A}$	$6.11\pm0.38^{ab,A}$	*0.035*
	P	*≤0.001*	*≤0.001*	*≤0.001*	*0.001*	*≤0.001*	
Chewiness ($N\ mm^{-1}$)	1	$6.57\pm0.32^{a,D}$	$9.65\pm0.58^{b,C}$	$11.88\pm0.55^{c,E}$	7.65 ± 1.16^{ab}	$11.23\pm0.43^{c,D}$	*≤0.001*
	2	$6.41\pm0.48^{a,CD}$	$7.29\pm0.30^{b,B}$	$9.57\pm0.51^{d,D}$	6.32 ± 0.63^{a}	$8.31\pm0.30^{c,C}$	*≤0.001*
	4	$5.38\pm0.24^{a,BC}$	$5.82\pm0.32^{ab,A}$	$7.24\pm0.46^{b,C}$	5.35 ± 0.30^{a}	$6.66\pm0.52^{ab,BC}$	*0.004*
	7	5.65 ± 0.26^{C}	5.42 ± 0.24^{A}	6.54 ± 0.38^{BC}	5.60 ± 0.36	6.33 ± 0.41^{B}	*n.s*
	9	4.00 ± 0.70^{A}	4.57 ± 0.78^{A}	5.69 ± 0.53^{AB}	5.14 ± 0.50	5.73 ± 0.23^{B}	*n.s*
	11	4.30 ± 0.38^{AB}	5.62 ± 0.40^{A}	5.09 ± 0.39^{A}	5.18 ± 0.52	4.71 ± 0.26^{A}	*n.s*
	15	3.84 ± 0.34^{A}	4.69 ± 0.31^{A}	4.67 ± 0.27^{A}	5.11 ± 0.47	4.07 ± 0.27^{A}	*n.s*
	P	*≤0.001*	*≤0.001*	*≤0.001*	*n.s*	*≤0.001*	
Resilience ($N\ mm^{-1}$)	1	0.26 ± 0.01^{C}	0.27 ± 0.01^{C}	0.27 ± 0.01^{B}	0.27 ± 0.01^{D}	0.28 ± 0.00^{C}	*n.s*
	2	0.26 ± 0.01^{C}	0.26 ± 0.01^{BC}	0.27 ± 0.01^{B}	0.26 ± 0.01^{CD}	0.28 ± 0.01^{C}	*n.s*
	4	0.25 ± 0.01^{BC}	0.25 ± 0.01^{BC}	0.25 ± 0.00^{A}	0.25 ± 0.01^{BCD}	0.26 ± 0.01^{BC}	*n.s*
	7	0.25 ± 0.01^{BC}	0.24 ± 0.01^{AB}	0.24 ± 0.01^{A}	0.24 ± 0.01^{AB}	0.25 ± 0.01^{AB}	*n.s*
	9	0.24 ± 0.00^{BC}	0.24 ± 0.01^{AB}	0.23 ± 0.01^{A}	0.24 ± 0.01^{AB}	0.25 ± 0.01^{AB}	*n.s*
	11	0.22 ± 0.00^{A}	0.24 ± 0.01^{AB}	0.23 ± 0.01^{A}	0.22 ± 0.01^{A}	0.24 ± 0.01^{A}	*n.s*
	15	0.23 ± 0.00^{AB}	0.22 ± 0.01^{A}	0.23 ± 0.00^{A}	0.23 ± 0.01^{A}	0.23 ± 0.01^{A}	*n.s*
	P	*0.003*	*0.003*	*≤0.001*	*≤0.001*	*≤0.001*	

Dietary treatments: CT: control diet. R25 and R50: diets including 25 and 50 g kg^{-1} raw microalgal biomass, respectively. H25 and H50: diets including 25 and 50 g kg^{-1} hydrolysed microalgae, respectively. Superscript uppercase letters indicate differences attributable to storage time within each treatment. Superscript lowercase 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

Table 7 Changes in fillet skin colour parameters during a 15-day cold storage (4 °C) period

Parameters	*Dpm*	Diets					*P*
		CT	R25	R50	H25	H50	
Lightness (L^*)	1	54.40 ± 0.78^{a}	59.43 ± 0.51$^{bc.C}$	60.93 ± 0.82$^{c.D}$	59.72 ± 0.78$^{bc.C}$	58.33 ± 1.00$^{b\ D}$	*≤ 0.001*
	2	54.60 ± 0.95^{a}	59.76 ± 0.63$^{c.C}$	57.80 ± 0.78$^{bc.C}$	57.46 ± 0.81$^{bc.BC}$	57.18 ± 0.66^{abC}	*≤ 0.001*
	4	54.18 ± 1.03	57.94 ± 0.46^{C}	57.10 ± 1.09^{BC}	56.99 ± 0.97^{B}	55.78 ± 0.94^{AB}	*n.s*
	7	55.61 ± 0.65	57.99 ± 0.64^{C}	55.86 ± 0.74^{ABC}	56.03 ± 0.57^{B}	57.57 ± 0.79^{C}	*n.s*
	9	54.75 ± 1.04	55.66 ± 0.79^{B}	55.25 ± 1.19^{ABC}	55.32 ± 1.11^{AB}	55.78 ± 0.94^{AB}	*n.s*
	11	55.03 ± 0.63	55.54 ± 0.84^{B}	54.44 ± 0.71^{AB}	53.17 ± 1.00^{A}	55.71 ± 0.76^{AB}	*n.s*
	15	55.18 ± 1.04	52.92 ± 0.67^{A}	54.19 ± 0.98^{A}	53.39 ± 0.51^{A}	55.27 ± 1.09^{A}	*n.s*
		n.s	*≤ 0.001*	*≤ 0.001*	*≤ 0.001*	*≤ 0.001*	
Greenness (a^*)	1	–0.06 ± 0.09$^{b.D}$	–0.49 ± 0.06$^{a.C}$	–0.55 ± 0.09$^{a.D}$	–0.67 ± 0.11$^{a.C}$	–0.66 ± 0.07$^{a.D}$	*≤ 0.001*
	2	–0.06 ± 0.08$^{c.D}$	–0.51 ± 0.06$^{b.C}$	–0.69 ± 0.06$^{ab.\ D}$	–0.97 ± 0.14$^{a.C}$	–0.80 ± 0.09$^{a.C}$	*≤ 0.001*
	4	–0.01 ± 0.17$^{c.D}$	–0.42 ± 0.14$^{b.C}$	–0.99 ± 0.15$^{a.C}$	–0.85 ± 0.13$^{a.C}$	–0.98 ± 0.12$^{a.C}$	*≤ 0.001*
	7	–0.80 ± 0.16$^{c.C}$	–1.55 ± 0.14$^{a.B}$	–1.64 ± 0.12$^{a.B}$	–0.97 ± 0.06$^{b.C}$	–1.47 ± 0.09$^{a.B}$	*≤ 0.001*
	9	–1.09 ± 0.15$^{b.BC}$	–1.48 ± 0.13$^{a.B}$	–1.61 ± 0.14$^{a.B}$	–1.50 ± 0.10$^{a.B}$	–1.53 ± 0.09$^{a.B}$	*0.021*
	11	–1.42 ± 0.08$^{b.B}$	–1.42 ± 0.15$^{b.B}$	–1.54 ± 0.14$^{b.B}$	–2.06 ± 0.10$^{a.A}$	–1.73 ± 0.17$^{ab.AB}$	*0.045*
	15	–1.85 ± 0.10^{A}	–1.87 ± 0.19^{A}	–1.88 ± 0.13^{A}	–1.96 ± 0.10^{A}	–1.91 ± 0.14^{A}	*n.s*
		≤ 0.001	*≤ 0.001*	*≤ 0.001*	*≤ 0.001*	*≤ 0.001*	
Yellowness (b^*)	1	7.00 ± 0.27$^{a.D}$	9.23 ± 0.39$^{c.F}$	9.75 ± 0.52$^{c.E}$	7.46 ± 0.28$^{ab.C}$	8.13 ± 0.21$^{b.F}$	*≤ 0.001*
	2	7.49 ± 0.26$^{a.D}$	8.43 ± 0.23$^{b.E}$	9.08 ± 0.29$^{c.E}$	7.26 ± 0.17$^{a.C}$	8.11 ± 0.25$^{b.F}$	*≤ 0.001*
	4	5.96 ± 0.18$^{a.C}$	7.49 ± 0.30$^{b.D}$	8.35 ± 0.26$^{c.E}$	5.84 ± 0.14$^{a.B}$	7.30 ± 0.33$^{b.E}$	*≤ 0.001*
	7	4.83 ± 0.16$^{a.B}$	4.56 ± 0.29$^{a.C}$	5.77 ± 0.28$^{bc.C}$	5.05 ± 0.18$^{ab.B}$	6.02 ± 0.10$^{c.D}$	*≤ 0.001*
	9	2.26 ± 0.15$^{b.A}$	3.40 ± 0.12$^{c.B}$	4.36 ± 0.15$^{d.B}$	1.76 ± 0.15$^{a.A}$	3.71 ± 0.23$^{c.C}$	*0.001*
	11	2.16 ± 0.16$^{b.A}$	3.39 ± 0.13$^{d.B}$	2.16 ± 0.19$^{b.A}$	1.53 ± 0.16$^{a.A}$	2.67 ± 0.21$^{c.B}$	*≤ 0.001*
	15	2.00 ± 0.16$^{bc.A}$	1.38 ± 0.19$^{a.A}$	2.26 ± 0.12$^{c.A}$	2.05 ± 0.11$^{bc.A}$	1.90 ± 0.16$^{b.A}$	*0.017*
		≤ 0.001	*≤ 0.001*	*≤ 0.001*	*≤ 0.001*	*≤ 0.001*	

Dietary treatments: CT: control diet. R25 and R50: diets including 25 and 50 g kg^{-1} raw microalgal biomass, respectively. H25 and H50: diets including 25 and 50 g kg^{-1} hydrolysed microalgae, respectively. Superscript uppercase letters indicate differences attributable to storage time within each treatment. Superscript lowercase 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

Not only total muscle lipid content, but also the qualitative profile of fatty acids was different among the experimental groups (Table 5). As mentioned above, the inclusion levels studied (up to 5%) seem too low to cause dramatic changes in dietary FA profile (Table 2), although it is remarkable that, even so, muscle n-3 PUFAs increased consistently in all the microalgae-containing experimental batches. The lower total lipid content in muscle, together with this increase in n-3 PUFA, could indicate that *N. gaditana* caused increased lipolysis of non-structural fatty acids such as SFAs and MUFAs and therefore, certain selective retention of structural fatty acids (n-3 PUFA). These results are in agreement with previous studies pointing out to such effect in fish supplemented with both macro and microalgae (Jafari et al. 2014; Vizcaíno et al. 2014; Sáez et al. 2020; 2022). These changes in FA profile yielded decreased atherogenic (AI) and thrombogenic (TI) indices in microalgae-fed animals (Table 5), what should be considered a favourable effect, since lower values for these indices are associated with decreased risk of platelet aggregation and of thrombus and atheroma formation (Ulbricht and Southgate 1991).

A key aspect that influences fish fillet shelf life under cold storage is microbial spoilage, namely that caused by psychrophilic bacteria. It is known that microalgae are rich in substances with potential antimicrobial activity, such as pigments, polyphenols, terpenoids, polysaccharides, among others. For instance, microalgal compounds have shown antimicrobial effects against some fish pathogenic bacteria both in vitro (Narasimhan et al. 2013) and in vivo (Magnoni et al. 2017). This potential of algal extracts might well be contemplated as natural additive for external application when it comes to preserving fresh fish, and thus, several studies have reported delayed growth of spoilage bacteria during cold storage of fish, this ending up in increased fish shelf life (Yarnpakdee et al. 2019; Stejskal et al. 2020; Sáez et al. 2021). In addition to the value of algal extracts as food additive intended for direct application on fish, they

Table 8 Changes in flesh colour parameters during a 15-day cold storage (4 °C) period

	Dpm	Diet					*P*
		CT	R25	R50	H25	H50	
Lightness (L*)	1	39.39 ± 0.54$^{a.A}$	39.02 ± 0.32$^{a.A}$	38.93 ± 0.45$^{a.A}$	39.03 ± 0.20$^{a.A}$	40.06 ± 0.50$^{b.A}$	*≤ 0.001*
	2	39.47 ± 0.21$^{a.A}$	39.28 ± 0.35$^{a.A}$	39.82 ± 0.44$^{a.AB}$	40.35 ± 0.29$^{b.B}$	41.78 ± 034$^{c.AB}$	*≤ 0.001*
	4	40.63 ± 0.31$^{ab.AB}$	40.46 ± 0.37$^{b.B}$	39.54 ± 0.37$^{a.AB}$	41.96 ± 0.39$^{c.C}$	39.74 ± 0.29$^{a.A}$	*≤ 0.001*
	7	43.96 ± 0.29$^{c.C}$	43.53 ± 0.28$^{c.C}$	40.43 ± 0.31$^{a.B}$	43.61 ± 0.26$^{c.D}$	42.45 ± 0.36$^{b.B}$	*≤ 0.001*
	9	42.78 ± 0.30$^{b.B}$	43.97 ± 0.31$^{c.C}$	41.58 ± 0.58$^{a.B}$	43.59 ± 0.34$^{c.D}$	41.51 ± 0.45$^{a.AB}$	*≤ 0.001*
	11	42.95 ± 0.27$^{a.B}$	43.66 ± 0.25$^{ab.C}$	42.92 ± 0.32$^{a.BC}$	44.21 ± 0.54^{bD}	42.64 ± 0.15$^{a.B}$	*≤ 0.001*
	15	43.75 ± 0.24$^{b.C}$	43.86 ± 0.41$^{b.C}$	46.22 ± 0.25$^{c.C}$	45.55 ± 0.29$^{c.E}$	42.45 ± 0.45$^{a.B}$	*≤ 0.001*
		≤ 0.001	*≤ 0.001*	*≤ 0.001*	*≤ 0.001*	*≤ 0.001*	
Greenness (a*)	1	–1.53 ± 0.08$^{a\ A}$	–1.44 ± 0.07^{bAB}	–1.64^{c} ± 0.13^{aA}	–1.45 ± 0.10^{bC}	–1.39 ± 0.07^{aABC}	*0.022*
	2	–1.74 ± 0.08$^{a.A}$	–1.40 ± 0.12$^{c.AB}$	–0.83 ± 0.06$^{d.D}$	–0.82 ± 0.05$^{d.D}$	–1.60 ± 0.11$^{b.A}$	*≤ 0.001*
	4	–1.05 ± 0.08$^{c.B}$	–1.52 ± 0.07$^{a.A}$	–1.18 ± 0.05^{bB}	–1.50 ± 0.11$^{a.C}$	–1.01 ± 0.09$^{c.D}$	*0.016*
	7	–1.62 ± 0.10$^{ab.A}$	–1.56 ± 0.09$^{b.A}$	–1.85 ± 0.13$^{a.A}$	–1.78 ± 0.08$^{a.AB}$	–1.53 ± 0.03$^{b.AB}$	*0.029*
	9	–1.04 ± 0.14$^{d\ B}$	–1.57 ± 0.12$^{b\ A}$	–1.74 ± 0.07$^{a\ A}$	–1.62 ± 0.07$^{ab\ ABC}$	–1.16 ± 0.10$^{c\ CD}$	*≤ 0.001*
	11	–1.23 ± 0.12$^{c.A}$	–1.02 ± 0.09$^{d.C}$	–0.87 ± 0.08$^{d.BC}$	–1.82 ± 0.06$^{a.A}$	–1.63 ± 0.17$^{b.A}$	*≤ 0.001*
	15	–1.18 ± 0.11$^{c.B}$	–1.69 ± 0.13$^{a.A}$	–1.12 ± 0.08$^{c.BC}$	–1.58 ± 0.06$^{ab.BC}$	1.31 ± 0.09$^{bc.BC}$	*0.001*
		≤ 0.001	*≤ 0.001*	*≤ 0.001*	*≤ 0.001*	*≤ 0.001*	
Yellowness (b*)	1	–0.57 ± 0.08$^{c.A}$	–0.76 ± 0.09$^{b.A}$	–0.62 ± 0.09$^{bc.A}$	–0.52 ± 0.08$^{c.A}$	–0.99 ± 0.10$^{a.A}$	*≤ 0.001*
	2	0.31 ± 0.06$^{c.B}$	–0.71 ± 0.08$^{ab.BC}$	–0.78 ± 0.06$^{a.A}$	–0.61 ± 0.06$^{b.B}$	–0.68 ± 0.07$^{b.BC}$	*≤ 0.001*
	4	0.53 ± 0.06$^{c.B}$	–0.80 ± 0.09$^{a.C}$	–0.60 ± 0.13$^{b.A}$	–0.87 ± 0.09$^{a.C}$	–0.84 ± 0.09$^{a.AB}$	*≤ 0.001*
	7	2.10 ± 0.14$^{d.C}$	0.78 ± 0.08$^{c.C}$	–0.76 ± 0.12$^{a.A}$	0.74 ± 0.04$^{c.BC}$	–0.51 ± 0.02$^{b.C}$	*≤ 0.001*
	9	1.86 ± 0.10$^{d.D}$	0.52 ± 0.03$^{a.B}$	0.85 ± 0.08$^{b.B}$	1.14 ± 0.12$^{c.D}$	1.11 ± 0.08$^{c.D}$	*≤ 0.001*
	11	2.75 ± 0.17$^{e.E}$	0.80 ± 0.07$^{b.C}$	1.13 ± 0.08$^{c.B}$	0.65 ± 0.05$^{a.BC}$	1.42 ± 0.10$^{d.E}$	*≤ 0.001*
	15	2.56 ± 0.20$^{c.E}$	1.41 ± 0.13$^{ab.D}$	2.28 ± 0.16$^{c.C}$	1.22 ± 0.11$^{a.D}$	1.59 ± 0.12$^{b.E}$	*≤ 0.001*
		≤ 0.001	*≤ 0.001*	*≤ 0.001*	*≤ 0.001*	*≤ 0.001*	

Dietary treatments: CT: control diet. R25 and R50: diets including 25 and 50 g kg^{-1} raw microalgal biomass, respectively. H25 and H50: diets including 25 and 50 g kg^{-1} hydrolysed microalgae, respectively. Superscript uppercase letters indicate differences attributable to storage time within each treatment. Superscript lowercase 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

have also been proposed as feed additives, with the aim of improving different physiological aspects of live fish. In other words, bioactive compounds of microalgae might be delivered to fish tissues via dietary supplementation. This strategy has been scarcely explored so far, although valuable studies have been carried out aimed at assessing several plant extracts in diets for different livestock species (Cui et al. 2018 in broilers; Cimmino et al. 2018 in goat kids; Menchetti et al. 2020 in rabbits), and also in aquacultured fish (Pinedo-Gil et al. 2019 in rainbow trout).

The results obtained in this study indicate that psychrophilic bacterial counts measured in control fish throughout the cold storage ranged from 3.80 to 9.97 log cfu/g after 1 and 15 dpm, respectively. TVC in fillets from *N. gaditana*-supplemented fish were lower than those of control fillets (Fig. 1), becoming differences significant for this parameter from 7 dpm onwards, regardless the dose and pre-treatment of the biomass. According to ICMSF (1986), the maximum acceptable limit of this parameter in fresh fish is 6 log CFU g^{-1}, which was exceeded in control fillets after 7 dpm, but after 9 dpm in *N. gaditana*-supplemented fish. Therefore, the shelf life of fillets was extended by 2 days in microalgae-supplemented fish. This suggests that antimicrobial compounds of the microalgal biomass have been delivered through the experimental feeds towards fish muscle and skin, reaching a concentration enough to delaying microbial growth. In contrast to the external addition of bioactive extracts, which effects are circumscribed to the outer layer of fish, their inclusion in diets has been hypothesized to reach active levels in cells due to efficient systemic distribution of biomolecules after intestinal absorption (Wu et al. 2022). However, algae have rarely been used for this purpose (De la Fuente-Vázquez et al. 2014 in lambs; Sáez et al. 2020 in flatfish). Therefore, there is still considerable scope for further research in this specific field.

Changes occurring in fish during *post-mortem* storage may lead to oxidative damage, not least in the lipid fraction, rich in PUFAs. Oxidative deterioration of lipids impacts

undesirably on its nutritional value, organoleptic properties, and shelf life, altogether affecting the commercial value of the product. In addition, it is also known that lipid oxidation products have negative repercussion on consumers's health (Domínguez et al. 2019). Although synthetic antioxidants are regarded as safe once approved by the regulatory agencies, however, public opinion is generally more prone to the utilization of natural antioxidants instead, and microalgae can play an important role in this regard (Coulombier et al. 2021).

In this work, oxidative status of fillet lipids at the beginning of the storage period was similar in all the experimental batches (about 2.7 mg MDA kg^{-1} muscle), within the limits of "perfect quality" for fish products (Cakli et al. 2006). As expected, storage time caused significant increase in this parameter, but those fish fed with microalgae-enriched diets showed consistently lower TBARS values from day 4 onwards, even taking into account the relatively low inclusion levels assayed. Control fillets reached the limit for good quality fish (5 mg MDA kg^{-1} muscle) before 7 dpm, whereas the rest of experimental batches didn't reach such value up to 9 dpm (Fig. 2). This effect is likely due to the fact that *N. gaditana* is acknowledged as a valuable source of substances with antioxidant capacity (Sáez et al. 2022), especially carotenoid pigments and phenolic compounds. The hydrolysis of the biomass has been reported to increase the antioxidant capacity of microalgae (Galafat et al. 2020; Sáez et al. 2022), likely owing to increased release of the inner antioxidant substances, and this seems to be the case in our study as well, according to the results shown in Fig. 2.

It is widely acknowledged that the proliferation of psychrophilic microorganisms during cold storage of fish is responsible for the generation of alkaline compounds, which increase muscle pH values. In the present study, pH values increased throughout storage time in all the experimental groups, although those animals fed with any of the microalgae-containing diets yielded lower muscle pH than controls from day 2 onwards. Given that microbial counts were also lower in fillets of fish fed with microalgae, it is likely that changes in pH also reflect the antimicrobial effect mentioned above. There is a close relationship between pH and the structural integrity of muscle proteins in fish flesh, and consequently, in their capacity to retain water molecules. In this regard, WHC measurements in our assay paralleled changes in muscle pH attributable to the experimental diets. Thus, WHC decreased more markedly during cold storage in CT group than in any of the rest of the experimental batches.

And it is indeed the structure of skeletal muscle, together with the properties of connective tissue, and also the lipid content, what is critical for the textural parameters of fillets. Specificically, fillet hardness is a decisive texture parameter, intrinsically linked to muscle protein integrity. In agreement with what was observed for WHC and pH, hardness was improved as a result of the inclusion of *N. gaditana* biomass in the diets (Fig. 4), also in a dose-dependent manner.

Although an evident lack of consensus can be found in the literature, the favourable influence of dietary algal biomass in fish texture parameters has been reported in several studies (Kousoulaki et al. 2016; Mosha et al. 2020; Sáez et al. 2020). It is also well known that decreased total lipid content in fish muscle is associated with improved firmness (Thakur et al. 2003; Lefevre et al. 2015), an effect also taking place in this study (Table 4), which could have contributed to some extent the improved firmness measured in fillets.

Skin and fillet colour of farmed fish are important aspects that determine the acceptation of the product by consumers (Makri et al. 2021), and consequently, the purchase decision. In general, even if subjective, the closer the coloration of fish fillets is to the caracteristics of wild specimens, the more the consumers associate the product with a more natural taste and a healthier product (Gouveia et al. 2002; Pulcini et al. 2020; Makri et al. 2021). Therefore, another expected goal of the aquaculture practice is to produce wild-like looking fish.

As mentioned above, microalgae are an important source of pigments acknowledged as valuable natural substances involved in fish coloration (Nakano and Wiegertjes 2020). In agreement, Ribeiro et al. (2017) reported a more vivid yellow colour in *S. aurata* fed with the diatom *Phaeodactylum tricornutum* rich in fucoxanthin. Also increased yellowish skin pigmentation has been described in gilthead seabream supplemented with synthetic or natural carotenoids (Pulcini et al. 2020). In line with these studies, our results also have shown increased b^* values (yellowness) in the skin of seabream fed with the experimental diets enriched with *N. gaditana*, compared to control fish. Also, a significant decrease in a^* values (more greenish) have been also observed on the skin, presumably due to the abundance of green pigments in *N. gaditana*, such as chlorophyll. These changes together led to a favourable effect on skin colour, since the appearance of fillets was more similar to wild specimens. Comparable results have been reported in previous studies carried out on European sea bass (*Dicentrarchus labrax*) fed with *Tisochrysis lutea* alone (Tibaldi et al. 2015) or in combination with *Tetraselmis suecica* (Cardinaletti et al. 2018). Galafat et al. (2020) in gilthead seabream and Kousoulaki et al. (2020) in Atlantic salmon also described improved skin pigmentation owing to the cyanobacterium *Arthrospira platensis* and the microalga *Schizochytrium limacinum*, respectively.

The interest of the modification in fish pigmentation owing to dietary changes is not limited to initial stages after slaughtering, but also on the evolution throughout the shelf life of the product. As expected, the intensity of the colour parameters decreased with increasing storage time, a

well-known phenomenon already reported in *S. aurata* (Ünal Şengör et al. 2019). However, it is remarkable that the skin of fish supplemented with the microalgae-enriched diets, overall, yielded consistently higher *L** and *b** values, and lower *a** values throughout the complete storage period than those measured in the control batch. All these factors contributed to an increased "visual quality" of the fish, what is crucial for the commercial value of fillets.

Contrary to what was expected, raw microalgae tended to increase skin pigmentation to a greater extent than hydrolysed biomass, although the differences were significant only for *b** parameter at the highest inclusion level (R50 *vs*. H50, with the exception of 7 dpm). Similar results were reported by Sáez et al. (2022) in juvenile *S. aurata*, a fact that was explained by the possible damage of carotenoids released from pre-treated microalgal cells owing to the further thermal treatment of the ingredient mixture during feed processing, which involves extrusion at high temperature (in the region of 110 ºC).

In addition to skin colour, flesh colour for a given fish species should also fulfil a set of characteristics which values meet the quality criteria expected by consumers. Therefore, any dietary treatment that might impair flesh appearance (e.g., brightness losses, abnormal tonalities, etc.) is considered unacceptable, even if other quality criteria (e.g., lipid oxidation, microbial counts, etc.) are improved.

In this context, the results obtained in this study indicate that none of the values for muscle colour parameters were significantly affected by the dietary treatment under the perspective of possible commercial depreciation. In particular, the parameter *a** wasn't influenced by the inclusion of *N. gaditana* in feeds (Table 8), this suggesting poor deposition of carotenoids in the muscle tissue of this species, in agreement with previous studies (Gouveia et al. 2002).

One of the typical undesirable effects of cold storage on white muscle colour characteristics of gilthead seabream is the increase in *b** parameter, which has been attributed to the accumulation of lipid oxidation products and free amino groups from proteins (Silva-Brito et al. 2021), that leads to yellowish tonality. This phenomenon has been clearly observed in control fillets (Table 8), in which *b** values increased markedly from the beginning of the storage. On the contrary, the fillets from fish fed with microalgae-containing feeds delayed such increase in *b** parameter, not least at the higher inclusion level assayed (5%), irrespectively of the pre-treatment of the biomass. The persistence of the differences with respect to controls throughout the complete assay roughly paralleled the results found for lipid oxidation of fillets (Fig. 2), which confirms the beneficial effects of the microalgal biomass on both quality parameters. In addition, the results for both microbial counts and lipid oxidation indicate that the inclusion of *N. gaditana* in feeds could extend the shelf life of refrigerated *S. aurata* fillets by two additional days, compared to the control lot, before reaching values beyond the acceptable range for these parameters.

As pointed out by Kiron et al. (2012), species-specific differences in composition among microalgae, inclusion level in diets, feed processing procedures, as well as dissimilar digestive and metabolic utilization of the microalgal biomass among fish species, should be taken into account before drawing any entirely valid conclusion on the potential effects of microalgal biomass included in feeds on the quality attributes of aquacultured fish.

Conclusions

The enrichment of the experimental feeds with *N. gaditana* at low level (up to 5%) yielded favourable effects on several objective quality parameters of *S. aurata* fillets obtained from fish slaughtered after a 42-day feeding trial. Specifically fatty acid profile, microbial counts, lipid oxidative status, textural and colour parameters of fillets resulted improved owing to the addition of the microalgal biomass in diets. These findings indicate that the shelf life of gilthead seabream fillets might well be extended by means of this dietary strategy. However, the effects were not significant in all cases. The fact that for most of the parameters yielded the best results at the highest inclusion level tested (5%) suggests a dose-dependent effect.

It was hypothesized that the cellulase pre-treatment would improve clearly most of parameters studied, as a result of increased release of the functional biomolecules from the inner compartment of the microalgal cells. However, no general conclusion can be drawn in this regard, given that although some parameters tended to improve (e.g. hardness and lipid oxidation), others did not yield such differences.

Although in vitro studies have found increased release of bioactive molecules due to physical and chemical microalgal disruption, however, under practical aquafeed processing conditions (i.e. extrusion involving high temperature and pressure), it cannot be assumed that such increased release of bioactive compounds implies necessarily enhanced biological effects on live fish. It is likely that thermolabile substances (e.g. carotenoids) could have been inactivated during feed manufacturing. Therefore, no general recommendation can be made with regard to the suitability of the biomass pre-treatment carried out here. This specific issue deserves further research.

Authors' contributions Sáez, M.I., Alarcón, F.J. and Martínez T.F. conceived and designed the experiments. Alarcón, F.J. and Galafat, A. prepared the aquafeeds. Galafat, A., Sáez, M.I. and Martínez, T.F. performed fish sampling. Arizcun, M., Chaves-Pozo, E. and Ayala, M.D. participated in sampling. Arizcun, M. and Chaves-Pozo, E. supervised

fish care and maintenance, and performed biometric measurements. Sáez, M.I., Galafat, A. and Alarcón, F.J. were in charge of proximal analysis. Suárez, M.D. performed and interpreted fatty acid analysis and texture profile analysis. Sáez, M.I., Martínez, T.F., Suárez, M.D., Galafat, A., Arizcun M., and Chaves-Pozo E. discussed the data. Sáez, M.I., Suárez, M.D. and Martínez, T.F drafted the manuscript. T.F. Martinez and M. Arizcun obtained the necessary funds for conducting the research. All authors critically revised and approved the manuscript.

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Data availability Data and material will be made available upon reasonable request.

Declarations

Competing interests The authors declare that they have no conflict of interest.

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