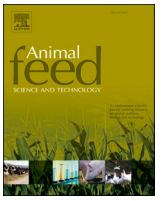
Animal Feed Science and Technology 314 (2024) 116017

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci



Incorporating sardine cooking water aromas into plant-based diets for European seabass: Effects on appetite regulation, growth and sensory properties of fish flesh

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ARTICLE INFO

Keywords:
Circular economy
European seabass
Aromas
Feed intake
Appetite stimulants
Palatability

ABSTRACT

Sardine cooking wastewaters are by-products of the canning industry with great potential for valorisation. We have hypothesized that they can be a source of aromas to enhance appetite when added to plant-based diets. The poor palatability of such diets often described in carnivorous species poses a recurring problem in fish farming, with harsh consequences on fish growth performance and flesh quality. Aromas from sardine cooking wastewaters were collected without processing (CW-A), processed through vacuum distillation (VD-A), or processed through liquid/liquid extraction with soybean oil (LLE-A) into plant-based diets. Each aroma was added to a plant-protein based diet for European seabass, at a concentration of 2 μg of 1-penten-3-ol/g diet, resulting in 3 experimental diets (CW, VD and LLE). A non-supplemented diet was used as a control. Each diet was assigned to triplicate fish groups (initial weight 95.7 g), that were hand-fed twice daily until apparent satiation in a recirculating saltwater system at 21 °C. After 18 weeks, fish growth performance and nutrient utilisation were evaluated. The expression in the brain of neuropeptides involved in feed intake regulation was also analysed. Moreover, flesh colour and texture were assessed instrumentally and by sensory analysis using a consumer panel. Fish fed LLE displayed a significantly higher feed intake than those fed CW which was correlated with an increased neuropeptide Y expression in the hypothalamus. However, LLE slightly hindered lipid metabolism, leading to lower available glucose and resulting in statistically similar final weights among diets. Despite variations in fillet hardness, the sensory panel revealed similar overall liking across all treatments. The findings indicate that aromas from sardine cooking wastewaters can

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https://doi.org/10.1016/j.anifeedsci.2024.116017
Received 5 January 2024; Received in revised form 29 February 2024; Accepted 29 May 2024
Available online 31 May 2024

modulate feed intake, but further refinement in processing or incorporation levels is required to potentiate their efficacy.

1. Introduction

Plant-based ingredients have been considered suitable options for replacing marine-based ingredients, such as fishmeal (FM) and fish oil (FO) in aquafeeds, since these have become scarce, expensive and environmentally unsustainable (Naylor et al., 2021). However, using plant ingredients as the main source of protein for farmed marine carnivorous species presents several challenges. Feed intake can be reduced when high levels of plant ingredients are included in aquafeeds, ultimately affecting fish growth, health or even sensorial properties. Torstensen et al. (2008) showed a reduced intake in Atlantic salmon (*Salmo salar*) smolts fed a diet with 80 % of plant proteins and 70 % vegetable oils, compared to a marine-based aquafeed, for the first three months of feeding these experimental diets. Likewise, in European seabass (*Dicentrarchus labrax*), Dias et al. (1997) observed a reduced feed intake when fed diets devoid of FM, relying on soy protein concentrates as main protein sources. Finally, Torrecillas et al. (2017) observed a reduction of feed intake of European seabass, after 90 days of feeding diets with 20 % FM and 6 % FO, compared with a diet with 58 % FM and 15 % FO. In fish farms, the feed can account for up to 40–60 % of the production costs (Prem and Tewari, 2020) and is also responsible for a large portion of the waste production in this sector, either due to uneaten feed or dietary digestibility issues (Kokou and Fountoulaki, 2018; Dauda et al., 2019). Therefore, both the sustainability of aquaculture and the quality of its end products will depend, among other factors, on improvements in diet formulation, a greater understanding of the fish behaviour and physiology, and dedicated research focused on improving feed intake and efficiency.

In fish, feed intake modulation depends on signals relating to energetic needs (homeostatic) and to the pleasant sensation of eating (hedonic) (Soengas et al., 2018). Integration of both hedonic and homeostatic signals occurs in the central nervous system, mainly in the hypothalamus and telencephalon regions (Lin et al., 2000; Delgado et al., 2017; Soengas, 2021; Díaz-Rúa et al., 2022). This integration results in the regulation of certain neuropeptides which directly influence feed intake. These neuropeptides can be anorexigenic (reduce feed intake), such as the cocaine and amphetamine-related transcript (CART) and the pro-opiomelanocortin (POMC) or orexigenic (increase feed intake), such as the agouti-related peptide (AgRP) and neuropeptide Y (NPY) (Comesaña et al., 2018a; Soengas, 2021). To increase feed intake of plant-based diets, which are generally less palatable than marine-based ones, modulation of the hedonic regulation of feed intake is needed, and one option for that is the use of feed attractants. The hedonic regulation of feed intake relies on sensorial and rewarding signals and it may even prevail over homeostatic regulation of intake, which can lead to feed consumption to levels that surpass those needed to cover all energy requirements (Comesaña et al., 2018b; Díaz-Rúa et al., 2022).

Several ingredients have been proposed as feed attractants for aquafeeds including amino acids (Kasumyan and Døving, 2003), peptides/hydrolysates (Chotikachinda et al., 2013), betaine (Mackie et al., 1980; Lim et al., 2016), nucleotides and nucleosides (Hossain et al., 2020). However, very little is yet known regarding other compounds such as alcohols, aldehydes and organic acids, which are known to promote food intake in mammals (Chen et al., 2017; Takács et al., 2018). Hence, the search for new feed attractants is still very relevant. In addition to its effectiveness, feed attractants must be economically and environmentally sustainable. The utilization of agri-food by-products as a source of such compounds holds the potential to contribute to circular economy policies (Utne-Palm et al., 2020).

In the canning industry, the production of one tonne of canned fish can generate as much as 9 m^3 of liquid waste. Notably, wastewaters produced during the cooking process are very rich in volatile compounds. However, these wastewaters must be processed prior to disposal to minimize environmental impact, which can be both complex and costly (Ferraro et al., 2013). Volatiles released during the cooking of fish in canning industries are currently not being recovered but once appropriately extracted, they have the potential to become valuable compounds, particularly when incorporated in aquafeeds. Beyond their potential as feed attractants, supplementing farmed fish diets with such volatiles can also help shape the fish's aroma profile, ultimately enhancing its appeal to consumers. However, to the best of our knowledge, no previous study assessed the impact of dietary inclusion of volatiles on the sensory profile of fish fillets. It is worth nothing that dietary changes have been reported to influence fish taste, flavour or texture (Schlüter et al., 1999; Luo et al., 2017; Ma et al., 2020). In addition, the replacement of marine ingredients by plant-based alternatives in diets may have implications for flesh quality (Fountoulaki et al., 2009). Therefore, the impact of diets with high levels of plant-based ingredients should always be considered.

Thus, the objective of this study was to explore the potential of incorporating aromas from sardine cooking wastewaters into plant-based diets for European seabass, a carnivorous species reliant on high levels of FM in its diet. The study aimed to investigate the effectiveness of these aromas as feed intake modulators and to assess their ability to enhance the organoleptic characteristics of fish fillets.

2. Materials and methods

2.1. Ethical issues

The experimental trial, previously approved by the CIIMAR ethical committee for Managing Animal Welfare (ORBEA-CIIMAR_18_2017), was performed by accredited scientists in laboratory animal science by the Portuguese Veterinary Authority (1005/92,

DGAV-Portugal, following FELASA category C recommendations) and conducted according to the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals for scientific purposes.

2.2. Aromas production

Sardine cooking wastewaters (A Poveira S.A., Laúndos, Portugal) were supplemented with a 1 % antioxidant extract (acorn extract) to minimize aroma deterioration. This cooking wastewater aroma, designated as CW-A, underwent additional processing as described by Resende et al. (2024). Initially, it was subjected to centrifugation to obtain an aqueous fraction, which was further treated though reverse osmosis. The resulting fraction underwent two distinct processes: liquid-liquid extraction with soybean oil, producing the LLE-A aroma, and vacuum distillation, resulting in the VD-A aroma (Resende et al., 2024).

2.3. Diet preparation

Four isonitrogeneous (52 % protein in dry matter, DM), isolipidic (18 % DM) and isoenergetic (22 kJ/g) diets were developed: a practical plant-based diet, with 12.5 % FM and 4 % FO, was defined as the control (CTRL); the three test diets (CW, LLE, VD) were obtained by adding each aroma to the CTRL (Table 1). The incorporation level of each aroma (CW-A, LLE-A, VD-A) into its corresponding diet (CW, LLE, VD) was set as to include the same amount of 1-penten-3-ol in all diets (2 μg/g diet). This compound was the most abundant in all extracted aromas and is responsible for the flavour of fresh marine products (Ganeko et al., 2008; Pereira et al., 2022a). All tested diets were formulated in conformity with European seabass nutrient requirements (National Research Council, 2011). All diets were formulated and extruded by SPAROS Lda. (Olhão, Portugal), using a pilot-scale twin-screw extruder (CLEXTRAL BC45, France), with a screw diameter of 55.5 mm, keeping temperature at 105–110 °C. The resulting extrudes, without oil, were oven-dried (OP 750-UF, LTE Scientifics, United Kingdom), for 3 h, at 60 °C. To avoid degradation of the extracts upon extrusion, each extract was added to the oil fraction and incorporated into extruded diets under vacuum conditions, in a Pegasus vacuum coater (PG-10VCLAB, DINNISEN, Netherlands). Experimental diets were then stored at 4 °C until use. Ingredients and proximate composition of diets are described on Table 1, reflecting the ingredient amounts considering the post-extrusion addition of the oils.

Table 1
Formulation and chemical composition of diets used in the growth trial.

	Diets			
	CTRL	CW	LLE	VD
Ingredients (g/kg)				
Fishmeal[a]	125.0	125.0	125.0	125.0
Soy protein concentrate[b]	250.0	250.0	250.0	250.0
Wheat gluten[c]	150.0	150.0	150.0	150.0
Corn gluten meal[d]	100.0	100.0	100.0	100.0
Soybean meal[e]	110.0	110.0	110.0	110.0
Wheat meal[f]	91.0	91.0	91.0	91.0
Vitamin and mineral premix[g]	5.0	5.0	5.0	5.0
DCP[h]	15.0	15.0	15.0	15.0
Fish oil[i]	40.0	40.0	40.0	40.0
Soybean oil [j]	114.0	114.0	76.5	114.0
CW-A (mL) [k]		12.00		
LLE-A [l]			37.5	
VD-A (mL) [m]				100.00
Chemical composition (%DM)				
Dry matter	95.24	94.53	95.08	94.53
Ash	6.52	6.51	6.57	6.51
Crude protein	52.19	52.28	52.15	52.28
Crude fat	18.12	17.87	17.58	17.87
Energy (kJ/g)	22.65	22.84	22.68	22.84
Phosphorus	0.85	0.86	0.87	0.86
1-Penten-3-ol (μg/g)	0.98	2.10	2.16	2.10

[a] Fishmeal NORVIK LT, Sopropêche, France (72 % crude protein, 7 % crude fat);
[b] Soy protein concentrate Soycomil®-P, ADM, Animal Nutrition ™, Netherlands (65 % protein, 0.7 % lipids);
[c] Wheat gluten composition: DM: 901 g kg^{-1}; protein: 838 g kg^{-1}; lipids: 16 g kg^{-1};
[d] Corn gluten feed from COPAM, Portugal (61 % crude protein, 6 % crude fat);
[e] Dehulled solvent extracted soybean meal, CARGILL, Spain (48 % crude protein, 2.2 % crude fat);
[f] Wheat meal from Casa Lanchinha Lda., Portugal (10.2 % protein, 1.2 % lipids);
[g] INVIVO 1 %, Premix for marine fish, PREMIX Lda, Portugal;
[h] Di-calcium phosphate;
[i] Sardine oil, Sopropêche, France;
[j] Henry Lamotte Oils, GmbH, Germany;
[k] Extract from sardine cooking wastewaters;
[l] Extract from sardine cooking wastewaters processed through liquid/liquid extraction;
[m] Extract from sardine cooking wastewaters processed through vacuum distillation.

2.4. Growth trial

Juvenile European seabass were transported from a commercial fish farm (Atlantik Fish Lda, Portugal) to the Fish Culture Experimental Unit of CIIMAR (Matosinhos, Portugal). Fish were quarantined for 2 weeks in a 2000 L tank included in a recirculating saltwater system (RAS) and fed a commercial diet (Aquasoja, Sorgal S.A.; 50 % crude protein, 20 % crude fat, as DM basis) once daily. Nitrogenous compounds ($NH_4^+ \leq 0.05$ mg L^{-1}; $NO_2^- \leq 0.5$ mg L^{-1}; $NO_3^- \leq 5$ mg L^{-1}) salinity (35 ‰), temperature (21 ± 1 °C), dissolved oxygen (>90 % saturation) and pH (7.5 ≤ pH ≤ 8.5) were frequently examined and kept at recommended levels for this species (Kır et al., 2019). A cycle of 12 h light/12 h dark was set as photoperiod.

After quarantine, fish were individually weighed (95.7 ± 13.5 g) and measured (total length, 20.4 ± 1.0 cm) and 12 homogeneous groups of 15 fish were randomly distributed (initial density of 7 kg m^{-3}) into 200 L fiberglass tanks within a RAS (10 L min^{-1} flow rate). Environmental conditions were kept as described for the quarantine period. Each diet was randomly assigned to triplicate groups of fish that were fed to apparent satiation, twice a day by hand, for 18 weeks. Feed intake was calculated by weighing the given feed and subtracting any leftover feed collected from the tanks. At the beginning of the trial, 5 fish from the initial stock were collected after a 24 h fasting period, sacrificed by anaesthetic overdose (0.5 mL L^{-1} of 2-phenoxyethanol, Sigma-Aldrich, MO, USA), and stored at −80 °C, for initial whole-body composition analysis. An intermediate sampling was conducted at 7 weeks, in which fish were bulk weighed to monitor weight gain and register feed consumption.

At the end of the 18-week period, two fish per tank were collected after a 6-h fasting, sacrificed by anaesthetic overdose (2-phenoxyethanol), and individually weighed (g) and measured (total length, cm). Blood was collected with heparinized syringes and centrifuged at 5000 g for 10 min at 4 °C. The plasma was stored at −80 °C until metabolites analysis. Hypothalamus and telencephalon were also collected and flash-frozen in dry ice prior to storage at −80 °C until molecular biology analysis. The instrumental quantification of colour and texture was carried out in fresh dorsal muscle samples from those fish. After a 48-h fasting period the remaining 13 fish per tank were collected and individually weighed (g) and measured (total length, cm). Three fish per tank were sacrificed by anaesthetic overdose (2-phenoxyethanol) and stored at −80 °C, for whole-body composition analysis, and 10 fish per tank were sacrificed by ice bath and transported in styrofoam boxes with ice to SenseTest, Lda facilities for sensory analysis.

2.5. Chemical analysis

To analyse whole-body composition, three fish from each tank were pooled and ground and dry matter was determined (105 °C, 24 h). Then, pooled samples were freeze-dried. Diets were ground prior to chemical analysis.

Ground and dried samples were analysed according to AOAC methods, as described in Resende et al. (2022). Briefly, dry matter (105 °C, 24 h), ash (combustion in a muffle furnace, Nabertherm L9/11/B170, Bremen, Germany; 550 °C, 6 h), crude protein (N × 6.25; Leco nitrogen analyser FP-528, Leco Corporation, St. Joseph, USA), crude fat (petroleum ether extraction; Soxtec™ 2055, FOSS, Höganäs, Sweden), gross energy (adiabatic bomb calorimeter; Werke C2000, IKA, Staufen, Germany) and phosphorus (acid digestion of ashes followed by quantification of phosphates using ammonium molybdate and absorbance reading at 820 nm) were determined in duplicate. Additionally, in aromatic extracts and diets, SPME/GC-MS (solid phase microextraction followed by gas chromatography-mass spectrometry) was performed for identification of the aroma compounds present in the extracts and quantification of 1-penten-3-ol, using the method described in Pereira et al. (2022a). A Shimadzu gas chromatograph (GCMS-QP2010) equipped with a WAX column (30 m x 0.25 mm i.d. x 0.25 μm) was used during the study. Ultrapure helium at 1 mL min^{-1} was used as the carrier gas. The oven temperature program was: 60 °C (held for 4 min), followed by an increase of 2 °C min^{-1} to 180 °C. The injector temperature was set at 200 °C, limited by the temperature of desorption indicated by the SPME fibre manufacturer. Detector temperature was set at 220 °C, ionization source at 200 °C and the ionization mode was electron impact with electron energy of 70 eV. A volume of 6 mL was then extracted by CAR/PDMS fibre during 15 min to 60 °C with and without stirring. The time of analyte desorption from the SPME fibre was fixed at 10 min. The injection was performed in the spitless mode for 2 min. After this time, the split ratio was set at 1:20 until the final of the chromatographic run. 2-nonanol was used as an internal standard for analysis of the sample. To obtain a good extraction of the tested compounds, 2 g of NaCl were added to all the analysed samples.

2.6. Metabolite's analysis

All liver samples were homogenised with 0.6 M perchloric acid and neutralized with 1 M potassium bicarbonate, as described in Basto et al. (2022). After centrifugation (10000 g, 4.5 min), the supernatant was collected and used for metabolites quantification. Plasma samples were deproteinized with 0.6 M perchloric acid, followed by neutralization with potassium bicarbonate and centrifugation at 13500 g for 4.5 min. Metabolites quantification was performed in triplicate on a microplate spectrophotometer (BioTek Synergy HT, Vermont, USA), using commercial kits (glucose, lactate, triglycerides, total lipids and cholesterol: Spinreact, Barcelona, Spain; non-esterified fatty acids, NEFA: Wako Chemicals, Neuss, Germany), adapted to microplate, as described in Velasco et al. (2021).

2.7. Quantification of mRNA abundance of intake-regulating neuropeptides

Whole hypothalamus or telencephalon were used for total RNA extraction with Trizol reagent (Invitrogen, Carlsbad, CA, USA), in accordance with the manufacturer's recommendations (with some modifications), followed by purification using a NZY Total RNA Isolation Kit (NZYTech, Lisbon, Portugal), as reported in (Ferreira et al., 2020). RNA purity and quantity was assessed with a DeNovix

DS-11FX spectrophotometer (Wilmington, DE, USA), considering the absorbance ratio 260:280 nm. If the A260/A280 ratio was in the range of 1.80–2.20, samples were deemed suitable for analysis. Afterwards, 1.5 μg of RNA was reverse transcribed to cDNA, using a NZY First-Strand cDNA Synthesis Kit (NZYTech, Lisbon, Portugal), according to the manufacturer's instructions.

Neuropeptide Y (*npy*), agouti-related peptide (*agrp2*), cocaine and amphetamine-related transcript (*cartpt2*) and pro-opio melanocortin (*pomca*) expression was evaluated by real time quantitative PCR (RT-qPCR), using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA), with the CFX384 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The forms of the neuropeptides assessed in this study were chosen based on reports by Basto et al. (2021); Basto et al. (2022), considering previous reports of their roles as either orexigens or anorexigens. All reactions were carried out with primer (forward and reverse) concentrations of 40 – 400 nM, plus 5 μL of Green Supermix and 2 μL of cDNA, totalling a reaction volume of 10 μL. The applied thermal cycling conditions were: 95 °C for 2 min, followed by 40 cycles of two steps – first, 95 °C for 5 s; second, primer annealing temperature (60–62 °C) for 28 s (Table 2). After the final PCR cycle, post-amplification dissociation curves were systematically monitored (60–95, 0.5 °C in each cycle), to warrant reaction specificity. PCR efficiency was analysed in serial 2-fold dilutions of cDNA using a sample pool of all experiments, with the CFX Maestro 2.3 software (Bio-Rad, Hercules, CA, USA). Values between 90 % and 115 % were considered suitable (the R^2 for all genes assessed was higher than 0.92). Samples were assessed in duplicate. The negative control was set as blanks without cDNA. Table 2 describes the primer sequence, annealing temperatures, PCR efficiencies and accession numbers for all analysed genes.

For housekeeping genes, ribosomal protein 40 S (*rps40*), elongation factor 1 alpha (*ef1α*) and beta-actin (*βact*) were considered. With the qbase+ software, the most stable genes or combination of genes were determined, according to the lowest M index and CV. The Pfaffl method was then employed for relative quantification of target gene transcripts (Pfaffl, 2001).

2.8. Instrumental texture and colour

Instrumental colour and texture were evaluated as described in Pereira et al. (2022b). Briefly, skin and muscle colour were analysed with a CR-400 chroma meter (Konica Minolta Inc., Osaka, Japan), with respect to CIE standard illuminant D65. A white plate reference standard (Minolta Co, Ltd., Osaka, Japan) was used for calibration of the apparatus. Colour parameters were determined by applying the colorimeter onto either skin or raw fillets from 6 fish per dietary treatment, in three points above the lateral line of each fillet. After flashing, *CIE-L* a*b** values were registered. Fillet texture was assessed using a TA.XT.plus Texture Analyser (Stable Micro Systems Inc., Godalming, United Kingdom) with a 5 kg load cell and a 2.0 mm diameter probe. Hardness (N), adhesiveness (J), springiness, cohesiveness, chewiness (J) and resilience measurements were acquired by double compression (constant speed and penetration depth of 1 mm s^{-1} 320 and 4.0 mm, respectively) on the thickest portion of each raw fillet. Penetration depth was set according to the maximum distance that did not produce fibre breakage.

2.9. Consumer profiling and liking

The "rate-all-that-apply" (RATA) methodology (Ares et al., 2014; Baião et al., 2022) was applied to evaluate consumer profiling of the seabass samples. The list of terms in the RATA ballot were generated by the research team, combining previous qualitative consumer studies with published studies from evaluation of samples performed by a trained panel (Montero et al., 2005; Mendes and Gonçalves, 2008; Makol et al., 2013). Table 3 describes the final list of sensory attributes used in the RATA ballot. Then, overall liking using the classic 9-point hedonic scale (Peryam and Pilgrim, 1957) was assessed with a panel, made of 75 regular consumers of fish (minimum of one fish meal per week), recruited from the sensory evaluation company Sense Test's consumer database (Vila Nova de Gaia, Portugal). They were mostly residents in the Oporto metropolitan area (north of Portugal). The company ensures data protection and confidentiality following the EU General Data Protection Regulation (EU 2016/679), along with an established internal code of conduct, guaranteeing informed consent. Sensory evaluation was performed in individual tasting booths at a special room equipped according to ISO 8589:2007 - Sensory analysis - General guidance for the design of test rooms.

Table 2
Oligonucleotide sequences, efficiency and annealing temperature used for relative mRNA quantification through RT-qPCR.

Gene	Primer Sequence	Annealing temperature (°C)	PCR Efficiency (%)	Accession number
agrp2	F: GGGCAGAGGACACAAAGAAA	H: 62	H: 109	HE660087
	R: TGTGACTTTCCTGTGGTGGA	T: 62	T: 97	
npy	F: ACGGAGGGATACCCGGTGAA	H: 60	H: 110	AJ005378
	R: GCTGAGTAGTACTTGGCCAGCTC	T: 60	T: 111	
cartpt2	F: CCGAACCTGACCAGCGAGAA	H: 62	H: 105	MZ441181
	R: GCTCCCCGACATCACACGTT	T: 60	T: 106	
pomca	F: CCGGTCAAAGTCTTCACCTC	H: 62	H: 103	AY691808
	R: ACCTCCTGTGCCTTCTCCTC			
β-act	F: CAAAGCCAACAGGGAGAAGATGA	H: 62	H:102	AJ537421
	R: ACCGGAGTCCATGACGATAC	T: 60	T: 104	
rps40	F: TGATTGTGACAGACCCTCGTG	H: 62	H:103	HE978789
	R: CACAGAGCAATGGTGGGGAT	T: 62	T: 91	
ef1α	F: AACTTCAACGCCCAGGTCAT	H: 60	H: 107	AJ866727.1
	R: CTTCTTGCCAGAACGACGGT	T: 62	T: 97	

For sensory evaluation, gut and scales of European seabass were removed. Heads, tails and fins were cut off and fish were divided into three slices (anterior, middle and posterior). Each slice was wrapped in microperforated aluminium foil and steamed in a pre-heated industrial forced convector oven Rational Combi-Master CM61, Rational AG, Germany), for 10 min at 100 °C. Each consumer evaluated slices from the same part of the fish (anterior, middle or posterior) across all samples.

The participants were asked to carry out a RATA evaluation with a list of 24 sensory attributes divided into four dimensions (Table 3): appearance (5), odour (5), texture/mouth-feel (7) and taste (7). Panellists were asked to mark the terms they thought applicable for describing samples and then to rate the intensity of each selected attribute, with a 5-point scale (from 1 = "slightly applicable" to 5 = "very applicable").

2.10. Calculations

Condition index (K) = 100 × final weight, g / (final length, cm)^3

Daily growth index (DGI) = 100 × [(final body weight, g)$^{1/3}$ – (initial body weight, g)$^{1/3}$]/ trial duration, d

Average body weight (ABW, kg) = (initial body weight, kg + final body weight, kg) / 2

Voluntary feed intake (VFI, g kg^{-1}d^{-1}) = dry nutrient intake, g / (ABW × trial duration, d)

Feed conversion ratio (FCR) = dry feed intake, g / weight gain, g

Hepatosomatic index (HSI) = liver weight, g / body weight, g × 100

Viscerosomatic index (VSI) = viscera weight, g / body weight, g × 100

Protein efficiency ratio (PER) = (final body weight, g – initial body weight, g) / (feed intake, g × protein amount in diets, %)

Nutrient gain (g kg^{-1} d^{-1}) = (final whole body nutrient content, g – initial whole body nutrient content, g) / (ABW × trial duration, d) × 100

Nutrient retention (%) = 100 × [(final body weight, g × final nutrient whole-body composition, %WW) – (initial body weight, g × initial nutrient whole-body composition, %WW)] / (feed intake, g × nutrient amount in diets, %)

2.11. Statistical analysis

All data were tested for normality and homogeneity of variances by Kolmogorov-Smirnov and Levene's tests, respectively, and adequately transformed if required. One-way ANOVA was applied to analyze data, using the SPSS (IBM SPSS Statistics 26, IL, USA) software. Whenever significant effects of treatments were detected, means were compared through the pairwise Tukey multiple comparison test. If data did not meet the assumptions of ANOVA, a Kruskal-Wallis test was carried out for each factor and the pairwise multiple comparison of mean ranks was performed to detect significant differences between groups. For the sensory analysis, a data set was created with the RATA applicability (0 = "not applicable"; 1 = "slightly applicable" to 5 = "very applicable"), as described in Baião et al. (2022)., RATA scores were calculated for each sample and term through the sum of the scores provided by the consumers who selected that term as applicable for that sample. A three-way ANOVA, considering dietary treatments, part of fish and consumer (set as

Table 3
List of sensory attributes used in the "rate-all-that-apply" ballot (original in Portuguese). Attributes were classified as either absent or present and if present, the respective intensity was rated using a 5-point structured scale (from 1 = "slightly applicable" to 5 = "very applicable").

Sensory dimension	Sensory attributes
Appearance (*Aparência*)	Moist (*húmido*), juicy (*suculento*), white flesh (*carne branca*), dark flesh in the middle (*carne escura no interior*), glossy flesh (*carne brilhante*)
Odour (*Odor*)	Fish smell (*a peixe*), sea smell (*a mar/maresia*), musty (*a mofo*), soft/weak (*suave/pouco intenso*), fresh (*fresco*)
Texture/Mouth-feel (*Textura*)	Slimy (*mole*), dry (*seca*), fibrous (*fibrosa*), juicy (*suculenta*), tender (*macia*), firm (*firme*), easy to flake (*fácil de lascar*)
Taste (*Sabor*)	Earthy (*a terra*), soft (*suave*), seabass characteristic (*típico a robalo*), intense (*intenso*), balanced (*equilibrado*), persistent (*persistente*), musty (*a mofo*)

random), was applied to identify which attributes were discriminating among samples ($p < 0.05$), followed by a pairwise multiple comparison test (Tukey HSD).

3. Results

3.1. Aromas and diets

The LLE-A displayed the highest amount of organic acids; VD-A had the highest concentrations of alcohols and ketones; finally, CW-A was richest in aldehydes (Fig. 1). Organic acids were not found in the CW-A and VD-A. Among the compounds present, 1-penten-3-ol was found to be the most abundant in all aromas. As expected, the supplemented diets showed identical levels of this compound, which were well above those observed on the CTRL diet (0.9 vs 2.1; Table 1).

3.2. Growth performance

The fish doubled their weight during the growth trial (Table 4). However, the experimental diets did not result in any statistically significant differences ($p>0.05$) in the final weight, length, condition index, or daily growth index. The LLE-fed fish had the highest VFI and FCR. It significantly differed from those fed the CW diet ($p=0.026$). However, there were no significant differences between the LLE diet and the CTRL and VD diets for these parameters. The CW-fed fish displayed the highest HSI, but they differed only from the LLE group ($p=0.020$). The PER followed a similar trend ($p=0.028$). No differences were observed for the VSI among the fish fed the different dietary treatments ($p>0.05$).

Feeding fish the experimental diets did not lead to any significant differences ($p>0.05$) regarding final whole-body composition or nutrient gain and retention (Table 4).

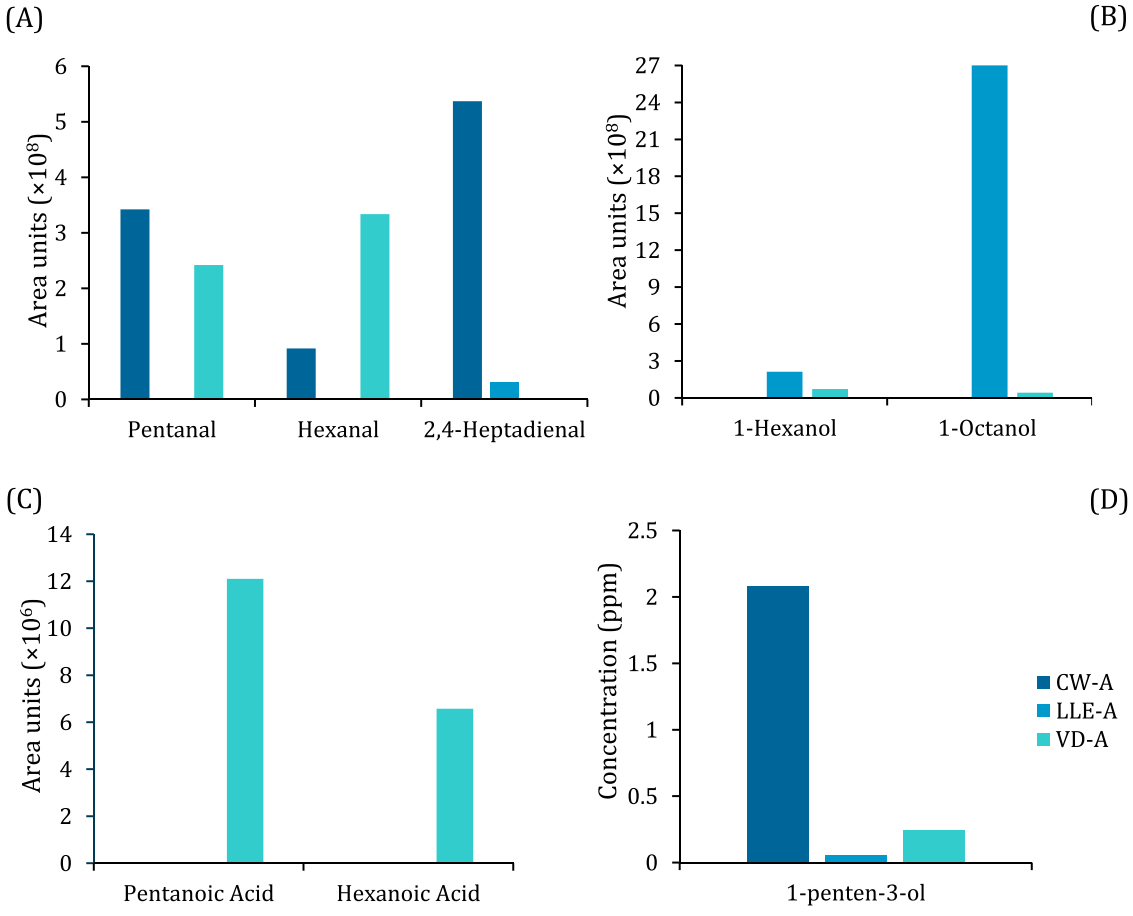

Fig. 1. Chemical profile of the different aromas (CW-A, LLE-A, VD-A): (A) main identified aldehydes; (B) main identified alcohols (excluding the marker compound); (C) main identified organic acids; (D) concentration of the compound set as marker, 1-penten-3-ol.

3.3. Metabolites in plasma and liver

Regarding metabolites concentration in plasma, only glucose resulted in significant differences (p=0.033) among diets (Table 5). The CTRL diet led to higher plasma glucose levels compared to the LLE diet. Lactate, NEFA, cholesterol and triglycerides remained unaffected by dietary treatments (p>0.05). In the liver, glucose level was highest in the LLE-fed fish, which differed significantly (p=0.014) from the CW group (Table 5). The same tendency was observed for triglycerides (p=0.025). No differences among fish fed the different diets were observed in terms of liver glycogen, NEFA and total lipids (p>0.05).

3.4. Neuropeptide mRNA abundance on brain tissues

There were no significant differences (p>0.05) in neuropeptide expression observed in either the hypothalamus or telencephalon (Fig. 2). However, in the hypothalamus, the LLE diet led to a higher *npy* expression compared to the CTRL and CW diets. The supplemented diets also appeared to slightly increase *agrp2* expression in the hypothalamus, although not significantly (p>0.05). Neuropeptide expression in the telencephalon showed more uniformity among diets. It was not possible to quantify *pomca* in the telencephalon due to its very low abundance. To assess the relationship between neuropeptide expression in both tissues and VFI, a Pearson correlation analysis was performed. The only significant correlation found was between *npy* expression in the hypothalamus and VFI, showing a positive correlation with a Pearson correlation coefficient of 0.762, and a p-value of 0.004.

3.5. Instrumentally assessed texture and colour properties

Table 6 depicts the instrumentally assessed colour properties of fish fillets and skin. Skin L^* values ranged from 38 to 41, a^* from −2.6 to −3.0 and b^* from 5.8 to 7.1. Regarding muscle colour, L^* values varied from 38 to 41, a^* from −1.8 to −2.2 and b^* from 3.0 to 5.1. There were no significant differences observed as a result of the dietary treatments (p>0.05).

Regarding fillet texture (Table 6), only hardness was significantly affected (p=0.006) by diets, with LLE leading to a lower hardness than CTRL; no other differences were observed among fillets from different dietary treatments (p>0.05).

Table 4
Growth performance indicators and final whole-body composition of European seabass fed the experimental diets for 18 weeks.

	Diet				*p*-value
	CTRL	**CW**	**LLE**	**VD**	
Initial weight (g)	95.53 ± 13.25	95.75 ± 13.01	95.78 ± 14.21	95.63 ± 13.95	0.994
Final weight (g)	209.46 ± 39.09	206.56 ± 35.95	205.27 ± 37.39	210.28 ± 35.59	0.910
Initial length (cm)	20.4 ± 0.9	20.4 ± 0.9	20.4 ± 1.1	20.4 ± 0.9	>0.999
Final length (cm)	25.8 ± 1.3	25.9 ± 1.3	25.8 ± 1.4	26.0 ± 1.3	0.793
K	1.21 ± 0.09	1.17 ± 0.08	1.19 ± 0.07	1.18 ± 0.07	0.309
DGI	1.05 ± 0.02	1.03 ± 0.01	1.02 ± 0.04	1.06 ± 0.01	0.222
VFI (g/kg/day)	7.68 ± 0.21^{ab}	7.46 ± 0.03^{b}	7.98 ± 0.03^{a}	7.93 ± 0.16^{ab}	0.029
FCR	1.34 ± 0.01^{ab}	1.32 ± 0.01^{b}	1.43 ± 0.07^{a}	1.37 ± 0.02^{ab}	0.026
HSI (%)	1.57 ± 0.26^{ab}	2.06 ± 0.38^{a}	1.51 ± 0.32^{b}	1.55 ± 0.28^{ab}	0.020
VSI (%)	7.71 ± 0.89	8.16 ± 1.12	7.24 ± 1.26	7.57 ± 1.40	0.608
PER	1.43 ± 0.01^{ab}	1.45 ± 0.01^{a}	1.35 ± 0.07^{b}	1.39 ± 0.01^{ab}	0.028
Final whole-body composition (%WW)					
Moisture	62.36 ± 0.81	64.00 ± 0.61	62.43 ± 0.76	62.36 ± 1.48	0.182
Ash	3.17 ± 0.23	3.30 ± 0.16	3.05 ± 0.31	2.94 ± 0.24	0.353
Protein	18.22 ± 0.41	18.32 ± 0.67	19.09 ± 0.30	18.72 ± 0.57	0.215
Fat	17.04 ± 1.00	15.05 ± 0.81	16.69 ± 0.61	17.27 ± 1.68	0.134
Energy (kJ/g)	9.77 ± 0.25	9.43 ± 0.09	9.75 ± 0.30	10.02 ± 0.36	0.144
Phosphorus	0.50 ± 0.03	0.52 ± 0.04	0.49 ± 0.06	0.46 ± 0.05	0.630
Gain (g or kJ/kg ABW/day)					
Dry matter	2.21 ± 0.12	2.00 ± 0.06	2.15 ± 0.12	2.22 ± 0.16	0.176
Protein	1.04 ± 0.03	1.03 ± 0.06	1.10 ± 0.05	1.10 ± 0.07	0.288
Fat	1.05 ± 0.12	0.83 ± 0.08	0.99 ± 0.08	1.08 ± 0.18	0.121
Energy	56.69 ± 3.66	52.16 ± 1.25	55.09 ± 4.60	59.69 ± 4.32	0.171
Phosphorus	0.020 ± 0.003	0.022 ± 0.004	0.019 ± 0.006	0.017 ± 0.005	0.684
Retention / consumption (%)					
Dry matter	28.76 ± 0.86	26.77 ± 0.71	26.91 ± 1.67	28.01 ± 2.15	0.361
Protein	25.98 ± 1.19	26.57 ± 1.77	26.57 ± 1.27	26.51 ± 1.59	0.950
Fat	75.48 ± 7.11	62.17 ± 5.99	70.64 ± 5.63	76.37 ± 13.06	0.233
Energy	32.56 ± 1.31	30.74 ± 0.60	30.49 ± 3.02	32.95 ± 2.19	0.379
Phosphorus	31.39 ± 4.84	34.27 ± 6.29	27.02 ± 7.65	25.42 ± 7.78	0.411

Values presented as mean ± SD (n=3 except for final weight, length and K, in which n=45). Different superscript letters indicate significant differences between dietary treatments. Abbreviations: ABW – average body weight; DGI – daily growth index; FCR – feed conversion ratio; HSI – hepatosomatic index; K – condition index; PER - protein efficiency ratio; VFI – voluntary feed intake; VSI – viscerosomatic index.

Table 5
Plasma and liver metabolite concentrations, in fish fed the experimental diets for 130 days, expressed in mmol L^{-1} and in μmol g $tissue^{-1}$, respectively.

	Diets				
	CTRL	**CW**	**LLE**	**VD**	***p*-value**
Plasma					
Glucose	6.418 ± 0.317[a]	5.340 ± 0.472[ab]	4.659 ± 0.364[b]	5.407 ± 0.257[ab]	0.033
Lactate	7.185 ± 0.259	5.822 ± 0.672	5.683 ± 0.867	6.295 ± 0.590	0.358
NEFA	0.982 ± 0.219	0.593 ± 0.211	0.367 ± 0.107	0.544 ± 0.151	0.218
Cholesterol	5.099 ± 0.532	4.943 ± 0.739	3.254 ± 0.569	5.195 ± 0.503	0.098
Triglycerides	14.518 ± 1.491	11.902 ± 2.916	11.282 ± 2.685	10.688 ± 2.158	0.684
Liver					
Glucose	22.04 ± 3.52[ab]	13.70 ± 1.79[b]	31.62 ± 4.18[a]	20.09 ± 3.98[ab]	0.014
Glycogen	99.32 ± 7.56	86.74 ± 3.94	92.23 ± 6.68	98.57 ± 5.84	0.447
NEFA	0.731 ± 0.310	0.176 ± 0.042	0.662 ± 0.108	0.853 ± 0.202	0.106
Triglycerides	3.07 ± 0.90[ab]	2.08 ± 0.45[b]	5.59 ± 1.08[a]	3.15 ± 0.51[ab]	0.025
Total lipids[1]	20.61 ± 4.02	18.11 ± 1.41	19.69 ± 3.81	23.50 ± 2.61	0.649

Values presented as mean ± SE (n=6). Different superscript letters indicate significant differences between dietary treatments. 1Values presented in mg g-1. Abbreviations: NEFA – non-esterified fatty acids.

3.6. Consumer sensory analysis

Samples across all dietary treatments displayed a similar sensory profile (Fig. 3), with no statistically significant differences detected in any of the attributes ($p>0.05$). Regarding appearance, the most selected attributes were *juicy, moist* and *white flesh.* As for odour, it was mostly perceived as being *soft* and *fresh*. The texture was generally described as *soft* and *easy to chip*, while the taste was perceived as *soft, balanced* and *characteristic of seabass*. Attributes like *musty* odour and taste, as well as *earthy* taste, were barely perceived. The liking of seabass samples was high and remained unaffected ($p>0.05$) by the dietary treatments (Fig. 4), with all treatments receiving over 93 % positive responses and a median overall liking score of 8 out of 9.

4. Discussion

The goal of this study was to evaluate the possibility of using aromas derived from sardine cooking wastewaters, obtained under a circular economy approach, as potential feed intake modulators for European seabass. Additionally, the study aimed to assess the impact of these aromas on the overall flesh quality of farmed fish. The ingredients used in this work were mostly rich in alcohols and aldehydes, which derive from the breakdown of sardine oil during the steaming process (Ferraro et al., 2013). To the best of the authors' knowledge, this represents the first study addressing aroma-rich extracts resulting from fish cooking wastewaters as potential feed intake modulators in a long-term trial.

The supplemented diets did not result in any discernible differences in terms of fish weight gain, as all treatments achieved similar final weights. This outcome was observed despite significant differences in VFI, indicating a change in the FCR. Fish fed the LLE diet displayed the highest VFI, but also showed the highest FCR and the lowest PER. These differences were statistically significant when compared to the CW group. Additionally, we observed a decrease in circulating glucose in the LLE group 6 h after feeding, which could potentially contribute to lower energy levels in comparison to the other groups. This reduction appears to trigger a cascade of signals that lead to an increase in *npy* levels in the hypothalamus, as evidenced by the positive correlation between VFI and *npy* expression in the hypothalamus. Consequently, this initiates an orexigenic response, stimulating appetite, and promoting food intake. Overall, these results suggest that the LLE diet might be influencing intake through a homeostatic regulatory mechanism. It is likely that the metabolism has been impaired, leading to suboptimal nutrient utilization. One plausible explanation could be that fish are less efficient at utilising lipids from the LLE diet, or that this diet hinders lipid digestion, causing fish to favour proteins as an energy source. This preference for proteins might explain the worse FCR and PER observed. To compensate these effects, fish increase their overall feed intake to maintain nutrient and energy homeostasis, ultimately leading to comparable nutrient gain and weight gain. Interestingly, the LLE group displayed the highest concentration of glucose in the liver, even though there were no statistically significant differences in liver glycogen. This suggests that the glycogen in the liver is being converted into glucose, which is then released into the bloodstream, potentially as an attempt to counteract the low plasmatic levels of glucose. The LLE diet also resulted in the highest levels of triglycerides in the liver, despite no significant impact on the total lipids content due to dietary treatments. This suggests a potential disturbance in lipid metabolism, raising the possibility that triglycerides may not be efficiently used as energy sources and are instead deposited in the liver. However, it is worth noting that the nutrient gain values reported in this study fall within the range previously documented in the literature for this species (Batista et al., 2020; Pereira et al., 2022b).

The LLE-A was derived using soybean oil, and previous research reported that soybean oil might lead to an increased FCR, possibly due to an unbalanced fatty acid profile when compared to fish oil (Trushenski et al., 2013; Emre et al., 2016). It is also plausible than certain compounds present in the LLE-A extract may be influencing fish metabolism, either directly or indirectly. For example, these compounds might be affecting microbiota diversity, which, in turn, can impact fish nutrient digestibility and utilization. In future research, it might be worthwhile to explore alternative oil-based vehicles for extraction, such as microalgae oil. Microalgae oil has been

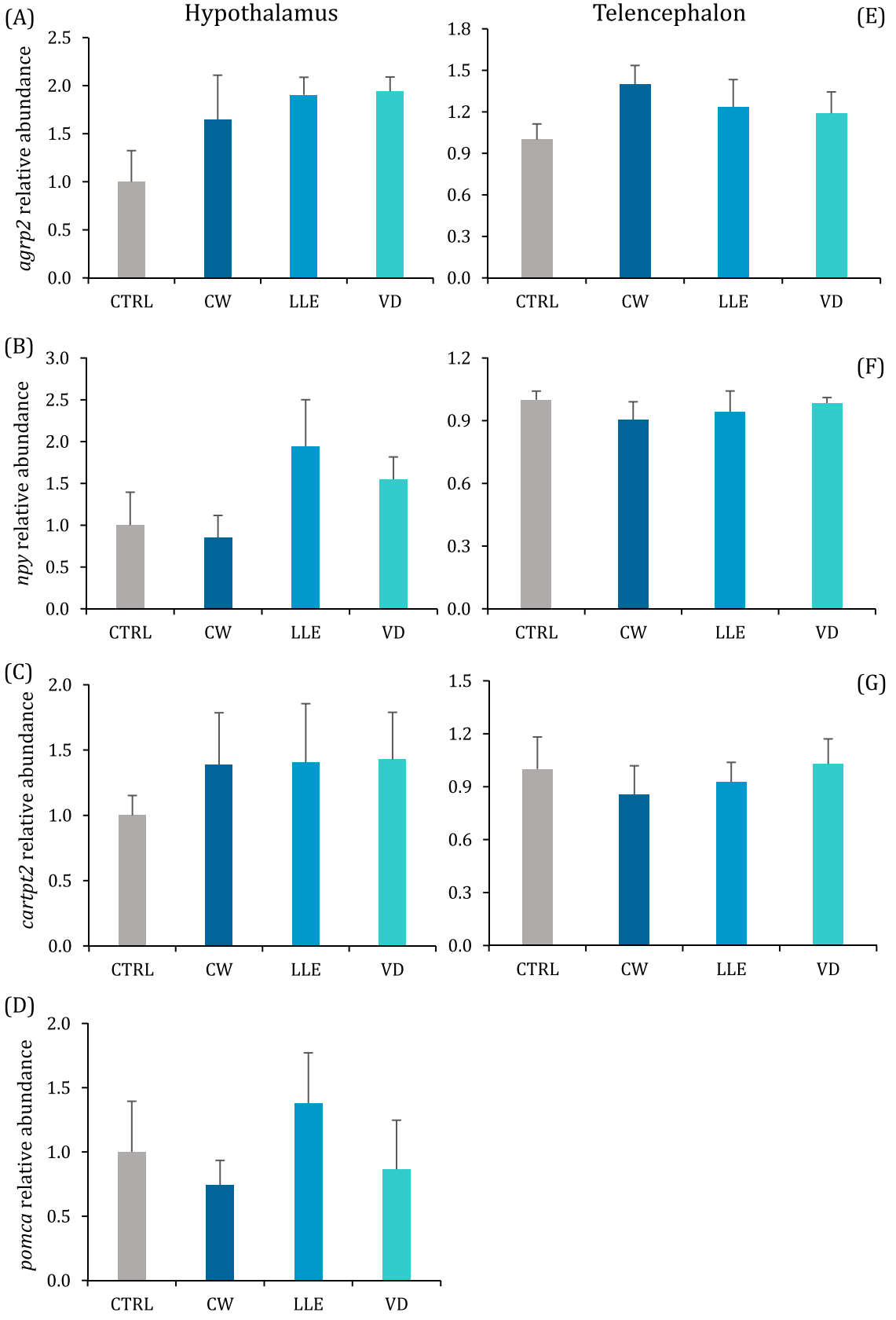

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Fig. 2. Relative mRNA abundance of neuropeptides related to the feed intake regulation in European seabass fed the experimental diets (CTRL, CW, LLE or VD) for 18 weeks, in the hypothalamus (A-D) or telencephalon (E-G). Values are presented as average + SE (n=6).

reported to possess valuable properties for aquafeeds, including high levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as antioxidants (Shah et al., 2018; Nagappan et al., 2021), which could open up the possibility of obtaining an extract that not only enhances VFI but also provides welfare-promoting properties for fish. However, the increase in the intake may also be a result of a hedonic regulation or, most likely, a combination of both homeostatic and hedonic influences. It has been reported that fish have high sensitivity to fatty acids (Soengas, 2014). While long and medium chain fatty acids appear to reduce feed intake (Velasco et al., 2020; Feng et al., 2022), short chain fatty acids may have the opposite impact (Zhang et al., 2020). Pentanoic acid and hexanoic acid, which are short-chain fatty acids, were exclusively found in the LLE-A extract, and these compounds might account for the slight increase on VFI. Additionally, aliphatic acids have previously been identified, through electrophysiological recordings, as having stimulatory properties in Japanese eel, Atlantic salmon and common carp. Notably, aliphatic acids with 3–5 carbons were found to be particularly effective in the latter species (Marui and Caprio, 1992; Kasumyan and Døving, 2003). Hexanal has previously been described as having a "fishy" odour (Giri et al., 2010), and LLE-A happens to contain the highest levels of this compound. In contrast, the CW-A presented high levels of pentanal and 2,4-heptadienal, which have been associated with a "rubber" and "rancid" odour (Venkateshwarlu et al., 2004). These compounds can function as off-flavours and discourage feed intake. The impact of aldehydes on fish taste response has received little attention. Jones (1990) reported that rainbow trout exhibited minimal to no response to aldehydes with 3–5 carbons. This study also found that *n*-hexanol and *n*-octanol were the most palatable alcohols. Both were found in LLE-A and VD-A. However, these compounds were not detected in the extract CW-A, and this diet also resulted in the lowest VFI; these two evidences could possibly be connected. However, it is worth noting that Jones (1990) performed the study using cotton pellets soaked in the compound solution rather than practical diets. The possibility of synergic or antagonistic effects within a complex mixture such as practical diets, which contain a multitude of nutrients beyond those found in the aromatic extracts, cannot be discarded. Additionally, fish fed CW displayed the lowest FCR and highest PER. Therefore, this might suggest that fish on this diet can efficiently utilize nutrients to meet their energetic needs, and since hedonic regulation is not triggered, the feed intake remains unaltered.

Despite variations in VFI observed among the supplemented diets, there were no significant differences found in the mRNA abundance of the neuropeptides involved in the regulation of feed intake. This lack of significant differences might be attributed to a potential habituation effect to the diets (Basto et al., 2021), as the analysis was performed after an 18-week feeding trial. Although a*grp* levels were slightly elevated in the hypothalamus for the animals on supplemented diets, this increase was not enough to lead to a significant increase in their feed intake compared to the control group. In addition, the orexigenic *npy* was slightly upregulated in the hypothalamus for the LLE diet compared to the CW diet. This partially explains the increased VFI observed in fish fed on the LLE diet. This relationship is further supported by the positive correlation found between *npy* expression and VFI. The neuropeptide responses in the telencephalon were slightly different compared to those in the hypothalamus, with less variation observed in response to diets. This suggests that the diet had a comparatively lesser impact on the telencephalon. A similar pattern was found by Comesaña et al. (2018a) when studying the effect of valine and leucine on the feed intake of rainbow trout. They found that feed intake was more closely associated with neuropeptide expression in the hypothalamus than in the telencephalon, highlighting the importance of the hypothalamus in regulating feed intake. It should be noted that *pomca* was not detected in European sea bass telencephalon by RT-qPCR, confirming previous observations by Basto et al. (2021).

Skin and fillet colour are very important during the purchase decision, as they are among the first sensory attributes consumers notice. When diets are rich in plant-based feed ingredients, there is a concern that the skin may appear darker or duller coloured, which consumers often associate with a product that is less fresh (Pleić et al., 2022). In this study, the dietary treatments did not influence skin nor fillet colour. This was expected, since the aromatic extracts did not possess significative pigmentation. The instrumental colour parameters observed in this study are in line with previous literature reports on European seabass fed diets rich in plant-based ingredients (Bonvini et al., 2018).

Additionally, the textural properties of the muscle play a crucial role in consumer acceptance of the product. These properties can be affected by both nutrient deposition in the muscle and the animals' growth performance, making them susceptible to dietary manipulations. In this work, only hardness was significantly affected by diets, with the control displaying a higher hardness than the LLE diet. However, this difference was not perceived by the consumer panel, as all samples were generally rated as *juicy*, *tender* and *easy to flake*. This discrepancy between instrumental and panel analysis can be attributed to the fact that instrumental analysis is conducted on raw fish, while sensory evaluation is based on cooked fish (Kotzamanis et al., 2020), making any minor differences undiscernible by consumers. Changes in fillet texture can be a attributed to factors like muscle cell density and fibre cross-sectional area (Matos et al., 2012). Conducting a histological assessment of the muscle could possibly shed some light on the reason behind the observed differences in this parameter. It should also be mentioned that the use of soybean oil as an extract medium might be a contributing factor. Previous studies reported a small decrease in fillet firmness in seabream when soybean oil was included in their diets (Izquierdo et al., 2003, 2005).

Regarding odour and flavour, no differences were detected by the consumer panel. This suggests that the aromas did not accumulate in the fish fillet and, as a result, did not impact the flavour and odour typically associated with European seabass. This is in agreement with the work of Iniesta et al. (2022), who reported that the dietary inclusion of essential oils, also rich in aroma compounds, did not affect gilthead seabream flavour as evaluated by a sensory panel. A similar situation occurred in pork meat, where the inclusion of essential oils from oregano, rosemary, garlic or ginger (all rich in aroma and flavour compounds) in pig diets did not lead to

Table 6
Instrumental colour and texture analysis of skin and fillets of fish fed the experimental diets. Values presented as mean ± SD (n=6).

	Diet				*p*-value
	CTRL	CW	LLE	VD	
Skin Colour					
*L**	38.93 ± 5.78	38.03 ± 2.45	41.16 ± 6.52	39.55 ± 6.44	0.797
*a**	-2.72 ± 0.73	-2.63 ± 0.44	-2.87 ± 0.28	-3.01 ± 0.75	0.685
*b**	5.89 ± 0.87	6.35 ± 0.91	7.06 ± 0.78	6.77 ± 1.30	0.216
Muscle colour					
*L**	40.75 ± 3.15	39.58 ± 0.98	38.44 ± 1.96	40.71 ± 1.33	0.190
*a**	-1.83 ± 0.26	-1.81 ± 0.45	-2.25 ± 0.45	-1.97 ± 0.40	0.282
*b**	5.08 ± 2.61	4.41 ± 1.05	3.08 ± 0.92	4.30 ± 1.61	0.130
Muscle texture					
Hardness (N)	0.914 ± 0.053^{a}	0.844 ± 0.085^{ab}	0.721 ± 0.078^{b}	0.745 ± 0.032^{ab}	0.006
Adhesiveness (J)	0.005 ± 0.003	-0.006 ± 0.009	-0.003 ± 0.015	0.007 ± 0.025	0.598
Springiness	1.042 ± 0.058	1.029 ± 0.075	1.091 ± 0.055	1.005 ± 0.055	0.813
Cohesiveness	0.541 ± 0.117	0.519 ± 0.046	0.514 ± 0.073	0.507 ± 0.062	0.888
Chewiness	0.520 ± 0.119	0.454 ± 0.070	0.406 ± 0.103	0.428 ± 0.086	0.229
Resilience	0.361 ± 0.046	0.365 ± 0.095	0.369 ± 0.091	0.359 ± 0.097	0.997

Different superscript letters indicate significant differences between dietary treatments.

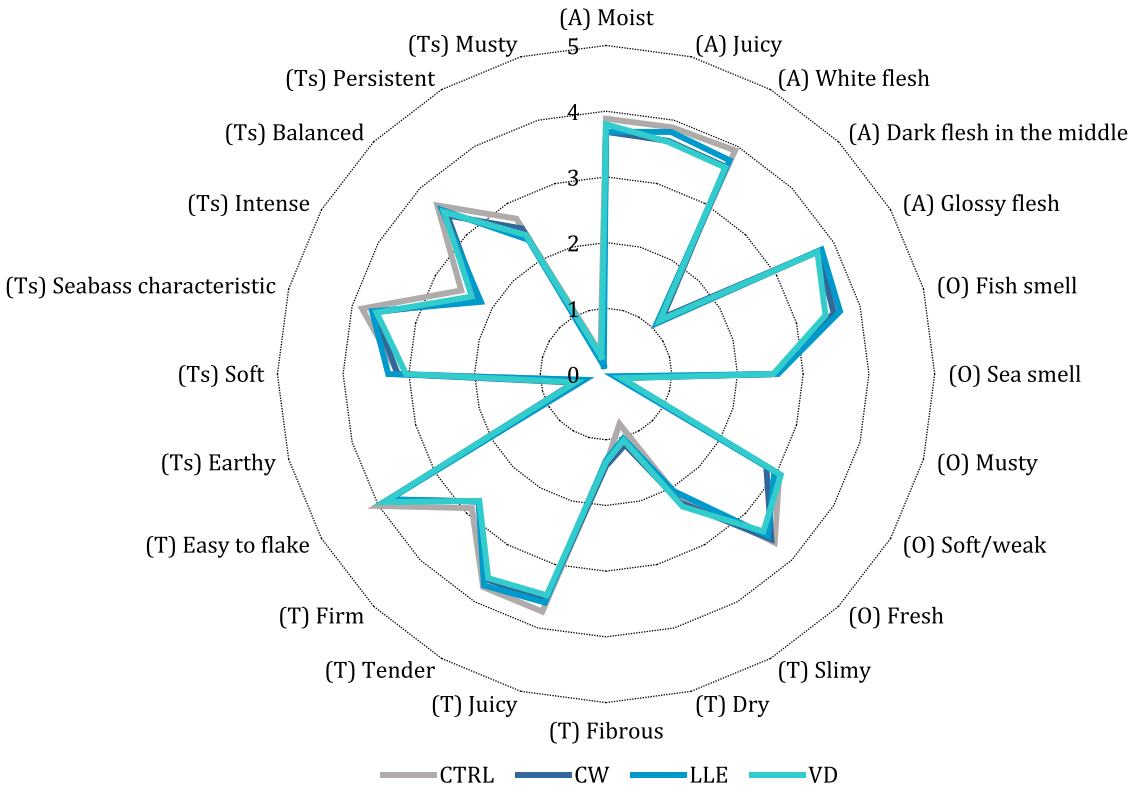

Fig. 3. Radar plot with average ratings of the sensory attributes (0 if unselected, otherwise rated on a scale from 1 to 5, with 1 = "slightly applicable" to 5 = "very applicable") of the fish fillets. Sensory attributes are ordered by sensory dimension: A - appearance, O – odour, T – texture and Ts – taste.

any noticeable differences in meat flavour or odour (Janz et al., 2007).

Moreover, consumer liking of all samples was high, with a median rating of 8, and all dietary treatments received over 90 % positive (6 – "slightly like or above") scores. This indicates that all tested diets produced fish of high quality for consumption, as perceived by consumers.

5. Conclusions

The diet LLE has resulted in a slight increase on fish voluntary feed intake (VFI) but has also elevated the feed conversion ratio

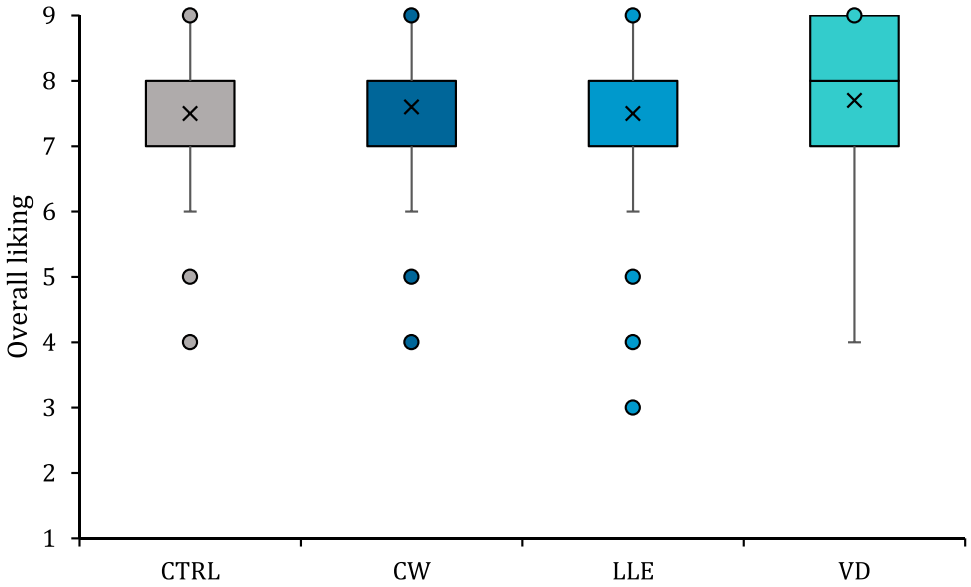

Fig. 4. Box and whisker plots of the distribution of overall liking ratings (9-point hedonic scale, from 1 to 9), by the consumer panel (n=70) of fish samples from different dietary treatments.

(FCR). The increase in intake was correlated with a higher expression of neuropeptide y in the hypothalamus. Moreover, these aromas did not affect other growth performance indicators, nor did they compromise the sensory profile of fish muscle. Overall, while this innovative approach holds promise, it is essential to conduct studies to determine the optimal inclusion levels of these aromas in diets through dosage evaluations to maximize their effectiveness.

Funding

This work was supported by Project "MobFood – Mobilizing scientific and technological knowledge in response to the challenges of the agri-food market" (POCI-01–0247-FEDER-024524), financed by ERDF, through PORTUGAL2020/COMPETE2020/ Lisb@2020. DR thanks Foundation for Science and Technology (FCT), Sense Test and the SANFEED programme and for her PhD grant (PD/BDE/150524/2019). Authors also acknowledge financial support from FCT, through programs UIDB/04423/2020 (CIIMAR), UIDB/05748/2020 and UIDP/05748/2020 (GreenUPorto).

CRediT authorship contribution statement

Luisa M.P. Valente: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Daniela Resende:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Validation, Visualization, Writing – original draft, Writing – review & editing. **Rui C. Lima:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization, Supervision. **Luís M. Cunha:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Manuela Pintado:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Carla Brazinha:** Resources, Methodology, Conceptualization, Supervision, Writing – review & editing. **Maria J. Pereira:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Cristina Velasco:** Conceptualization, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. **Célia Rocha:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Tiago Sá:** Formal analysis, Investigation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Ares, G., Bruzzone, F., Vidal, L., Cadena, R.S., Giménez, A., Pineau, B., Hunter, D.C., Paisley, A.G., Jaeger, S.R., 2014. Evaluation of a rating-based variant of check-all-that-apply questions: rate-all-that-apply (RATA). Food Qual. Prefer. 36, 87–95. https://doi.org/10.1016/j.foodqual.2014.03.006.

Baião, L.F., Rocha, C., Lima, R.C., Valente, L.M.P., Cunha, L.M., 2022. Development of a Rate-All-That-Apply (RATA) ballot for sensory profiling of sea urchin (*Paracentrotus lividus*) gonads. Food Res. Int. 153, 110976 https://doi.org/10.1016/j.foodres.2022.110976.

Basto, A., Valente, L.M.P., Conde-Sieira, M., Soengas, J.L., 2021. Central regulation of food intake is not affected by inclusion of defatted *Tenebrio molitor* larvae meal in diets for European sea bass (*Dicentrarchus labrax*). Aquaculture 544, 737088. https://doi.org/10.1016/j.aquaculture.2021.737088.

Basto, A., Valente, L.M.P., Soengas, J.L., Conde-Sieira, M., 2022. Partial and total fishmeal replacement by defatted *Tenebrio molitor* larvae meal do not alter short- and mid-term regulation of food intake in European sea bass (*Dicentrarchus labrax*). Aquaculture 560, 738604. https://doi.org/10.1016/j.aquaculture.2022.738604.

Batista, S., Pereira, R., Oliveira, B., Baião, L.F., Jessen, F., Tulli, F., Messina, M., Silva, J.L., Abreu, H., Valente, L.M.P., 2020. Exploring the potential of seaweed *Gracilaria gracilis* and microalga *Nannochloropsis oceanica*, single or blended, as natural dietary ingredients for European seabass *Dicentrarchus labrax*. J. Appl. Phycol. 32, 2041–2059. https://doi.org/10.1007/s10811-020-02118-z.

Bonvini, E., Parma, L., Badiani, A., Fontanillas, R., Gatta, P.P., Sirri, F., Nannoni, E., Bonaldo, A., 2018. Integrated study on production performance and quality traits of European sea bass (*Dicentrarchus labrax*) fed high plant protein diets. Aquaculture 484, 126–132. https://doi.org/10.1016/j.aquaculture.2017.10.041.

Chen, M., Chen, X., Nsor-Atindana, J., Masamba, K.G., Ma, J., Zhong, F., 2017. Optimization of key aroma compounds for dog food attractant. Anim. Feed Sci. Technol. 225, 173–181. https://doi.org/10.1016/j.anifeedsci.2016.12.005.

Chotikachinda, R., Tantikitti, C., Benjakul, S., Rustad, T., Kumarnsit, E., 2013. Production of protein hydrolysates from skipjack tuna (*Katsuwonus pelamis*) viscera as feeding attractants for Asian seabass (*Lates calcarifer*). Aquac. Nutr. 19, 773–784. https://doi.org/10.1111/anu.12024.

Comesaña, S., Velasco, C., Conde-Sieira, M., Míguez, J.M., Soengas, J.L., Morais, S., 2018b. Feeding stimulation ability and central effects of intraperitoneal treatment of L-leucine, L-valine, and L-proline on amino acid sensing systems in rainbow trout: Implication in food intake control. Front. Physiol. 9, 1209. https://doi.org/10.3389/fphys.2018.01209.

Comesaña, S., Velasco, C., Ceinos, R.M., López-Patiño, M.A., Míguez, J.M., Morais, S., Soengas, J.L., 2018a. Evidence for the presence in rainbow trout brain of amino acid-sensing systems involved in the control of food intake. Am. J. Physiol. Regul. Integr. Comp. Physiol. 314, R201–R215. https://doi.org/10.1152/ajpregu.00283.2017.

Dauda, A.B., Ajadi, A., Tola-Fabunmi, A.S., Akinwole, A.O., 2019. Waste production in aquaculture: sources, components and managements in different culture systems. Aquac. Fish. 4, 81–88. https://doi.org/10.1016/j.aaf.2018.10.002.

Delgado, M.J., Cerdá-Reverter, J.M., Soengas, J.L., 2017. Hypothalamic integration of metabolic, endocrine, and circadian signals in fish: involvement in the control of food intake. Front. Neurosci. 11, 354. https://doi.org/10.3389/fnins.2017.00354.

Dias, J., Gomes, E.F., Kaushik, S., 1997. Improvement of feed intake through supplementation with an attractant mix in European bass fed plant-protein rich diets. Aquat. Living Resour. 10 https://doi.org/10.1051/alr:1997043.

Díaz-Rúa, A., Chivite, M., Comesaña, S., Conde-Sieira, M., Soengas, J.L., 2022. The opioid system in rainbow trout telencephalon is probably involved in the hedonic regulation of food intake. Front. Physiol. 13 https://doi.org/10.3389/fphys.2022.800218.

Emre, Y., Kurtoğlu, A., Emre, N., Güroy, B., Güroy, D., 2016. Effect of replacing dietary fish oil with soybean oil on growth performance, fatty acid composition and haematological parameters of juvenile meagre, *Argyrosomus regius*. Aquac. Res. 47, 2256–2265. https://doi.org/10.1111/are.12677.

Feng, H., Peng, D., Liang, X.-F., Li, J., Luo, H., Tang, S., Chai, F., 2022. Intracerebroventricular injection with octanoic acid activates hypothalamic fatty acid sensing systems and regulates appetite in Chinese perch *Siniperca chuatsi*. Fish. Sci. 88, 83–90. https://doi.org/10.1007/s12562-021-01570-1.

Ferraro, V., Carvalho, A.P., Piccirillo, C., Santos, M.M., L. Castro, P.M., E. Pintado, M., 2013. Extraction of high added value biological compounds from sardine, sardine-type fish and mackerel canning residues — a review. Mater. Sci. Eng. C. 33, 3111–3120. https://doi.org/10.1016/j.msec.2013.04.003.

Ferreira, M., Larsen, B.K., Granby, K., Cunha, S.C., Monteiro, C., Fernandes, J.O., Nunes, M.L., Marques, A., Dias, J., Cunha, I., Castro, L.F.C., Valente, L.M.P., 2020. Diets supplemented with Saccharina latissima influence the expression of genes related to lipid metabolism and oxidative stress modulating rainbow trout (Oncorhynchus mykiss) fillet composition. Food Chem. Tox. 140, 111332 https://doi.org/10.1016/j.fct.2020.111332.

Fountoulaki, E., Vasilaki, A., Hurtado, R., Grigorakis, K., Karacostas, I., Nengas, I., Rigos, G., Kotzamanis, Y., Venou, B., Alexis, M.N., 2009. Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.); effects on growth performance, flesh quality and fillet fatty acid profile: recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. Aquaculture 289, 317–326. https://doi.org/10.1016/j.aquaculture.2009.01.023.

Ganeko, N., Shoda, M., Hirohara, I., Bhadra, A., Ishida, T., Matsuda, H., Takamura, H., Matoba, T., 2008. Analysis of volatile flavor compounds of sardine (*Sardinops melanostica*) by solid phase microextraction. J. Food Sci. 73, S83–S88. https://doi.org/10.1111/j.1750-3841.2007.00608.x.

Giri, A., Osako, K., Ohshima, T., 2010. Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. Food Chem. 120, 621–631. https://doi.org/10.1016/j.foodchem.2009.10.036.

Hossain, M.S., Koshio, S., Kestemont, P., 2020. Recent advances of nucleotide nutrition research in aquaculture: a review. Rev. Aquac. 12, 1028–1053. https://doi.org/10.1111/raq.12370.

Iniesta, C., Zapata, E., Firmino, J.P., Peñaranda, I., Egea, M., Linares, M.B., Garrido, M.D., 2022. Effect of the dietary supplementation based on essential oils on the quality of gilthead seabream. Aquac. Res. 53, 2567–2574. https://doi.org/10.1111/are.15796.

Izquierdo, M.S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L., Rosenlund, G., 2003. Dietary lipid sources for seabream and seabass: growth performance, tissue composition and flesh quality. Aquac. Nutr. 9, 397–407. https://doi.org/10.1046/j.1365-2095.2003.00270.x.

Izquierdo, M.S., Montero, D., Robaina, L., Caballero, M.J., Rosenlund, G., Ginés, R., 2005. 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 250, 431–444. https://doi.org/10.1016/j.aquaculture.2004.12.001.

Janz, J.A.M., Morel, P.C.H., Wilkinson, B.H.P., Purchas, R.W., 2007. Preliminary investigation of the effects of low-level dietary inclusion of fragrant essential oils and oleoresins on pig performance and pork quality. Meat Sci. 75, 350–355. https://doi.org/10.1016/j.meatsci.2006.06.027.

Jones, K.A., 1990. Chemical requirements of feeding in rainbow trout, *Oncorhynchus mykiss* (Walbaum); palatability studies on amino acids, amides, amines, alcohols, aldehydes, saccharides, and other compounds. J. Fish. Biol. 37, 413–423. https://doi.org/10.1111/j.1095-8649.1990.tb05872.x.

Kasumyan, A.O., Døving, K.B., 2003. Taste preferences in fishes. Fish Fish 4, 289–347. https://doi.org/10.1046/j.1467-2979.2003.00121.x.

Kır, M., Sunar, M.C., Gök, M.G., 2019. Acute ammonia toxicity and the interactive effects of ammonia and salinity on the standard metabolism of European sea bass (*Dicentrarchus labrax*). Aquaculture 511, 734273. https://doi.org/10.1016/j.aquaculture.2019.734273.

Kokou, F., Fountoulaki, E., 2018. Aquaculture waste production associated with antinutrient presence in common fish feed plant ingredients. Aquaculture 495, 295–310. https://doi.org/10.1016/j.aquaculture.2018.06.003.

Kotzamanis, Y., Kumar, V., Tsironi, T., Grigorakis, K., Ilia, V., Vatsos, I., Brezas, A., van Eys, J., Gisbert, E., 2020. Taurine supplementation in high-soy diets affects fillet quality of European sea bass (*Dicentrarchus labrax*). Aquaculture 520, 734655. https://doi.org/10.1016/j.aquaculture.2019.734655.

Lim, L.-S., Chor, W.-K., Tuzan, A.D., Shapawi, R., Kawamura, G., 2016. Betaine is a feed enhancer for juvenile grouper (*Epinephelus fuscoguttatus*) as determined behaviourally. J. Appl. Anim. Res. 44, 415–418. https://doi.org/10.1080/09712119.2015.1091329.

Lin, X., Volkoff, H., Narnaware, Y., Bernier, N.J., Peyon, P., Peter, R.E., 2000. Brain regulation of feeding behavior and food intake in fish. Comp. Biochem. Phys. A 126, 415–434. https://doi.org/10.1016/S1095-6433(00)00230-0.

Luo, J.-B., Feng, L., Jiang, W.-D., Liu, Y., Wu, P., Jiang, J., Kuang, S.-Y., Tang, L., Tang, W.-N., Zhang, Y.-A., Zhou, X.-Q., 2017. Physical and flavor characteristics, fatty acid profile, antioxidant status and nrf2-dependent antioxidant enzyme gene expression changes in young grass carp (*Ctenopharyngodon idella*) fillets fed dietary valine. PLOS ONE 12, e0169270. https://doi.org/10.1371/journal.pone.0169270.

Ma, X.-Z., Feng, L., Wu, P., Liu, Y., Kuang, S.-Y., Tang, L., Zhou, X.-Q., Jiang, W.-D., 2020. Enhancement of flavor and healthcare substances, mouthfeel parameters and collagen synthesis in the muscle of on-growing grass carp (*Ctenopharyngodon idella*) fed with graded levels of glutamine. Aquaculture 528, 735486. https://doi.org/10.1016/j.aquaculture.2020.735486.

Mackie, A.M., Adron, J.W., Grant, P.T., 1980. Chemical nature of feeding stimulants for the juvenile Dover sole, *Solea solea* (L.). J. Fish. Biol. 16, 701–708. https://doi.org/10.1111/j.1095-8649.1980.tb03749.x.

Makol, A., Torrecillas, S., Vaquero, A., Rincon, L., Ginés, R., Izquierdo, M., 2013. Deposition of conjugated linoleic acid in market size sea bass (*Dicentrarchus labrax*) and its effects on performance, composition and fillet sensory and texture attributes. Aquac. Nutr. 19 https://doi.org/10.1111/anu.12025.

Marui, T., Caprio, J., 1992. Teleost gustation. In: Hara, T.J. (Ed.), Fish Chemoreception. Chapman & Hall, Winnipeg, Canada, pp. 171–198.

Matos, E., Gonçalves, A., Bandarra, N., Colen, R., Nunes, M.L., Valente, L.M.P., Dinis, M.T., Dias, J., 2012. Plant proteins and vegetable oil do not have detrimental effects on post-mortem muscle instrumental texture, sensory properties and nutritional value of gilthead seabream. Aquaculture 358-359, 205–212. https://doi.org/10.1016/j.aquaculture.2012.07.009.

Mendes, R., Gonçalves, A., 2008. Effect of soluble CO_2 stabilisation and vacuum packaging in the shelf life of farmed sea bream and sea bass fillets. Int. J. Food Sci. Tech. 43, 1678–1687. https://doi.org/10.1111/j.1365-2621.2008.01737.x.

Montero, D., Robaina, L., Caballero, M.J., Ginés, R., Izquierdo, M.S., 2005. Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: a time-course study on the effect of a re-feeding period with a 100% fish oil diet. Aquaculture 248, 121–134. https://doi.org/10.1016/j.aquaculture.2005.03.003.

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

National Research Council, 2011. Nutrient requirements of fish and shrimp. The National Academies Press, Washington, DC.

Naylor, R.L., Hardy, R.W., Buschmann, A.H., Bush, S.R., Cao, L., Klinger, D.H., Little, D.C., Lubchenco, J., Shumway, S.E., Troell, M., 2021. A 20-year retrospective review of global aquaculture. Nature 591, 551–563. https://doi.org/10.1038/s41586-021-03308-6.

Pereira, M.J., Grosjean, O., Pintado, M., Brazinha, C., Crespo, J., 2022a. Clean technologies for production of valuable fractions from sardine cooking wastewaters: an integrated process of flocculation and reverse osmosis. Clean. Technol. 4, 276–295. https://doi.org/10.3390/cleantechnol4020016.

Pereira, R., Costa, M., Velasco, C., Cunha, L.M., Lima, R.C., Baião, L.F., Batista, S., Marques, A., Sá, T., Campos, D.A., Pereira, M., Jesus, D., Fernández-Boo, S., Costas, B., Pintado, M., Valente, L.M.P., 2022b. Comparative analysis between synthetic vitamin E and natural antioxidant sources from tomato, carrot and coriander in diets for market-sized *Dicentrarchus labrax*. Antioxidants 11, 636. https://doi.org/10.3390/antiox11040636.

Peryam, D.R., Pilgrim, F.J., 1957. Hedonic scale method of measuring food preferences. Food Technol. 11, 9–14.

Pleić, I.L., Bušelić, I., Messina, M., Hrabar, J., Žuvić, L., Talijančić, I., Žužul, I., Pavelin, T., Anđelić, I., Pleadin, J., Puizina, J., Grubišić, L., Tibaldi, E., Šegvić-Bubić, T., 2022. A plant-based diet supplemented with *Hermetia illucens* alone or in combination with poultry by-product meal: one step closer to sustainable aquafeeds for European seabass. J. Anim. Sci. Biotechnol. 13, 77. https://doi.org/10.1186/s40104-022-00725-z.

Prem, R., Tewari, V.K., 2020. Development of human-powered fish feeding machine for freshwater aquaculture farms of developing countries. Aquac. Eng. 88, 102028 https://doi.org/10.1016/j.aquaeng.2019.102028.

Resende, D., Costas, B., Sá, T., Golfetto, U., Machado, M., Pereira, M., Pereira, C., Marques, B., Rocha, C.M.R., Pintado, M., Valente, L.M.P., 2022. Innovative swine blood hydrolysates as promising ingredients for European seabass diets: impact on growth performance and resistance to *Tenacibaculum maritimum* infection. Aquaculture 561, 738657. https://doi.org/10.1016/j.aquaculture.2022.738657.

Resende, D., Pereira, M.J., Sá, T., Brazinha, C., Pintado, M., Valente, L.M.P., Velasco, C., 2024. Production of aroma-rich extracts from sardine cooking wastewaters: Exploring their potential for modulating feed intake in European seabass. Waste Biomass Valoriz. [Accepted for publication].

Schlüter, S., Steinhart, H., Schwarz, F.J., Kirchgessner, M., 1999. Changes in the odorants of boiled carp fillet (*Cyprinus carpio* L.) as affected by increasing methionine levels in feed. J. Agric. Food Chem. 47, 5146–5150. https://doi.org/10.1021/jf9902604.

Shah, M.R., Lutzu, G.A., Alam, A., Sarker, P., Kabir Chowdhury, M.A., Parsaeimehr, A., Liang, Y., Daroch, M., 2018. Microalgae in aquafeeds for a sustainable aquaculture industry. J. Appl. Phycol. 30, 197–213. https://doi.org/10.1007/s10811-017-1234-z.

Soengas, J.L., 2014. Contribution of glucose- and fatty acid sensing systems to the regulation of food intake in fish. A review. Gen. Comp. Endocrinol. 205, 36–48. https://doi.org/10.1016/j.ygcen.2014.01.015.

Soengas, J.L., 2021. Integration of nutrient sensing in fish hypothalamus. Front. Neurosci. 15, 653928 https://doi.org/10.3389/fnins.2021.653928.

Soengas, J.L., Cerdá-Reverter, J.M., Delgado, M.J., 2018. Central regulation of food intake in fish: an evolutionary perspective. J. Mol. Endocrinol. 60, R171–R199. https://doi.org/10.1530/jme-17-0320.

Takács, S., Musso, A.E., Gries, R., Rozenberg, E., Borden, J.H., Brodie, B., Gries, G., 2018. New food baits for trapping house mice, black rats and brown rats. Appl. Anim. Behav. Sci. 200, 130–135. https://doi.org/10.1016/j.applanim.2017.11.011.

Torrecillas, S., Robaina, L., Caballero, M.J., Montero, D., Calandra, G., Mompel, D., Karalazos, V., Kaushik, S., Izquierdo, M.S., 2017. Combined replacement of fishmeal and fish oil in European sea bass (Dicentrarchus labrax): production performance, tissue composition and liver morphology. Aquaculture 474, 101–112. https://doi.org/10.1016/j.aquaculture.2017.03.031.

Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbø, R., Hemre, G.I., Fontanillas, R., Nordgarden, U., Hevrøy, E.M., Olsvik, P., Berntssen, M.H.G., 2008. Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. Aquaculture 285, 193–200. https://doi.org/10.1016/j.aquaculture.2008.08.025.

Trushenski, J., Mulligan, B., Jirsa, D., Drawbridge, M., 2013. Sparing fish oil with soybean oil in feeds for white seabass: effects of inclusion rate and soybean oil composition. N. Am. J. Aquac. 75, 305–315. https://doi.org/10.1080/15222055.2012.720650.

Utne-Palm, A.C., Bogevik, A.S., Humborstad, O.-B., Aspevik, T., Pennington, M., Løkkeborg, S., 2020. Feeding response of Atlantic cod (*Gadus morhua*) to attractants made from by-products from the fishing industry. Fish. Res. 227, 105535 https://doi.org/10.1016/j.fishres.2020.105535.

Velasco, C., Conde-Sieira, M., Comesaña, S., Chivite, M., Díaz-Rúa, A., Míguez, J.M., Soengas, J.L., 2020. The long-chain fatty acid receptors FFA1 and FFA4 are involved in food intake regulation in fish brain. J. Exp. Biol. 223, jeb227330. https://doi.org/10.1242/jeb.227330.

Velasco, C., Conde-Sieira, M., Comesaña, S., Chivite, M., Míguez, J.M., Soengas, J.L., 2021. Role of the G protein-coupled receptors GPR84 and GPR119 in the central regulation of food intake in rainbow trout. J. Exp. Biol. 224, jeb242360. https://doi.org/10.1242/jeb.242360.

Venkateshwarlu, G., Let, M.B., Meyer, A.S., Jacobsen, C., 2004. Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. J. Agric. Food Chem. 52, 311–317. https://doi.org/10.1021/jf034833v.

Zhang, H., Ding, Q., Wang, A., Liu, Y., Teame, T., Ran, C., Yang, Y., He, S., Zhou, W., Olsen, R.E., Zhang, Z., Zhou, Z., 2020. Effects of dietary sodium acetate on food intake, weight gain, intestinal digestive enzyme activities, energy metabolism and gut microbiota in cultured fish: Zebrafish as a model. Aquaculture 523, 735188. https://doi.org/10.1016/j.aquaculture.2020.735188.