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Sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) head oils recovered by microwave-assisted extraction: Nutritional quality and biological properties

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ABSTRACT

Microwave-assisted extraction (MAE) and Soxhlet extraction (SE) were used to obtain oil from European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) heads. The MAE technique allowed the recovery of more than 50% of the total lipid content for both fish by-products in less than 11 min extraction. Based on their fatty acid composition, all fish head oils presented a healthy lipid profile and were found to be a good source of docosahexaenoic acid (DHA, 11–14%). Different lipid quality indices also revealed their cardiovascular protective potential. Oils obtained by MAE showed higher antibacterial and antifungal effects against food pathogens than those extracted by SE. Cellular antioxidant activity (29–35% oxidation inhibition) and anti-inflammatory potential *via* NO production inhibition (IC_{50} = 14–21 μg/mL) were evaluated using murine macrophages cells (RAW 264.7). The highest cytotoxic effect (GI_{50} = 38–46 μg/mL) of fish head oils was observed against breast cancer cells (MCF-7). These results showed that sea bass and gilthead sea bream heads could be exploited for the production of oil with nutritional and bioactive properties in line with circular bioeconomy concepts.

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1. Introduction

The use of fish by-products throughout the world for different purposes, including human consumption is not new. In 1903, million tons of cod heads were used for the elaboration of feed and fertilizer in Norway, while fish skin has traditionally been used for clothing, bags, and carrier packs in different Nordic countries (Rustad et al., 2011). Cleaned fish stomach and fried fish milt in Asia as well as fish liver in Eastern Europe have been usually consumed (Rustad et al., 2011). Changes in the lifestyle of the population in developed countries have led to an increased demand for fish fillets and other fish products instead of whole fish. In this context, the manufacture of filleted, salted, smoked, and canned products generates 4.7 million tons of fish by-products each year in Europe (Lopes et al., 2015).

European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) are amongst the main finfish species farmed in the European Union (EU) (The EU Fish Market, 2020). Their consumption is very popular in Mediterranean countries since ancient times, where they are currently being

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traded as whole fish gutted head-off. After industrial processing, the heads of sea bass and sea bream represent about 19% and 17% of the total fish weight, respectively (Valcarcel et al., 2020). The increasing transformation of both fish species means a greater amount of heads shortly, which is an opportunity for their valorization by the fish processing industry.

The nutritional characterization of several by-products from sea bass and sea bream have been recently reported (Munekata et al., 2020; Pateiro et al., 2020; Valcarcel et al., 2020). The heads were found to contain 29–34% and 39–46% fat (dry weight) for sea bass and sea bream, respectively. The extracted oils presented similar fatty acids composition for both fish species, showing a healthy lipid profile. However, there are no studies in the literature regarding the potential biological activities of oils from sea bass and sea bream side stream materials.

Since valorization and upcycling are future-oriented and environmentally friendly concepts, sustainable technologies must be used for the production of value-added products from underutilized natural resources (Chemat et al., 2020). In this sense, several non-conventional techniques have been investigated to recover valuable compounds from seafood processing by-products (Bruno et al., 2019). Among them, microwave-assisted extraction (MAE) is considered a fast and efficient technique due to the microwave energy effect on the sample (Llompart et al., 2018). Theoretically, microwaves increase the temperature of the sample cells, causing the disruption of their membranes and the consequent transfer of compounds into the solvent (Alfio et al., 2021; Llompart et al., 2018). For instance, MAE was successfully applied for the extraction of chitosan from shrimp by-products, increasing the extraction yield and reducing the extraction time compared to conventional methods (Bruno et al., 2019). In a previous study, MAE was optimized to obtain oil with nutritional and bioactive properties from salmon backbones, heads, and viscera using the response surface methodology (de la Fuente et al., 2022). The results showed a good percentage of oil recovery in less than 15 min of extraction. In addition, the obtained oils presented a healthy lipid profile as well as relevant biological activities.

The objective of this study was to recover oil from European sea bass and gilthead sea bream heads by applying the optimal extraction conditions previously established for salmon heads. The fatty acid composition of the obtained oils was evaluated as an indicator of their nutritional quality, also verified by different lipid quality indices. The antibacterial, antifungal, antioxidant, anti-inflammatory, and cytotoxic activities of fish head oils were also studied to provide valuable information for the future valorization of these fish processing side streams. The properties of sea bass and sea bream head oils extracted by MAE were compared with those extracted by the traditional Soxhlet method.

2. Material and methods

2.1. Standards and reagents

The commercial antibacterial ampicillin, methicillin, and streptomycin were acquired from Fisher Scientific (Janseen Pharmaceutical, Belgium) while the commercial antifungal ketoconazole was provided by Frilabo (Porto, Portugal). The standards ellipticine, dexamethasone, and quercetin, as well as the fatty acid methyl ester (FAME) reference standard mixture 37 (standard 47885-U), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Analytical grade reagents were used for all experiments. Sulfuric acid (98%), n-hexane (95%), diethyl ether, methanol, and toluene were purchased from Fisher Scientific (Leicestershire, UK). Roswell Park Memorial Institute medium (RPMI 1640), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), L-glutamine, penicillin (100 U/mL)/streptomycin (100 mg/mL) solution, Hank's balanced salt solution (HBSS), and trypsin–EDTA (ethylenediaminetetraacetic acid) were provided by Hyclone (Logan, Utah, USA). Malt Extract Broth (MEB) and Muller-Hinton Broth (MHB) were from Biolab® (Budapest, Hungary) while Blood Agar (Sheep blood 7%) was from LiofilChemsrl (Roseto d. Abruzzi (TE), Italy). 2′,7′-Dihydrodichlorofluorescein diacetate (DCFH-DA), 2,2′-azobis(2-amidinopropane) dihydrochloride (APPH), tris(hydroxymethyl) aminomethane (Tris), trichloroacetic acid (TCA), dimethyl sulfoxide (DMSO), lipopolysaccharide (LPS), and sodium nitrate were also from Sigma-Aldrich. *p*-Iodonitrotetrazolium chloride (INT) and sodium sulfate were provided by Panreac Applichem (Barcelona, Spain). Water was treated through a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.2. Fish material and sample preparation

Farmed European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) were obtained in a local market in Valencia (Spain) on different days in February and April 2019. According to the commercial label, these are species cultivated in the Mediterranean area. The sea bass came from Spain and the sea bream from Greece. They were immediately transported from the market to the laboratories of the University of Valencia under refrigerated conditions. For each fish species, all individuals were dissected as a simulation of fish processing for human consumption, obtaining different by-products (including heads). Next, each type of by-product was processed individually (freezing, freeze-drying, grinding, and storage at −25 °C) as described in previous studies (de la Fuente et al., 2021a, 2021b). Regarding the samples of fish heads, freeze-dried heads of seabass and seabream were transported from the University of Valencia to the Mountain Research Center (CIMO, Bragança, Portugal) under refrigeration conditions for oil extraction and characterization.

2.3. Microwave-assisted extraction (MAE) and Soxhlet extraction (SE)

Oil extraction was performed in a microwave extractor (Nμ Tech, NuWav-Uno, Sonilex, West Bengal, India) equipped with a circulating cool-water reflux system, manual electromagnetic stirrer, and time controller. Microwave power conditions (max. 1000 W) and temperature overshoot were also controlled by the internal Intelli-System. The extraction conditions were established according to the values of microwave power (50.0 W), extraction time (10.8 min) and solid-to-liquid ratio (80 g/L) previously optimized for obtaining oil from salmon heads (de la Fuente et al., 2022). A total volume of solvent was fixed at 50 mL. After the extraction process, samples were filtered and hexane solvent was completely removed using a rotary vacuum evaporator (Hei-VAP Silver 4, Schwabach, Germany) at 40 °C. Obtained oils were stored at − 25 °C until subsequent characterization assays. At least five extractions were carried out for each fish head sample.

At the same time, the conventional Soxhlet extraction (SE) was used as a reference method for total lipid extraction. Head sample (5 g) and n-hexane (250 mL) were introduced in a laboratory Soxhlet extractor (Behr Labor Technik™, Düsseldorf, Germany), where the oil extraction was conducted at 80 °C for 6 h. After finishing the extraction time, solvent removal and oil storage were made as in the MAE process. All extractions were carried out at least in duplicate.

For all subsequent assays, both the optimal oils (MAE) and the oils obtained by SE were used at least in duplicate.

2.4. Oil yield determination

The amount of oil from sea bass and sea bream heads was calculated gravimetrically by applying the following formula:

$$\text{Oil yield (\%)} = (\text{weight of extracted oil} / \text{weight of fish head}) \times 100 \quad (1)$$

The percentage recovery values were expressed in dry weight (dw) due to the use of freeze-dried fish head samples for both extraction processes (SE and MAE).

2.5. Evaluation of nutritional properties of fish head oils

2.5.1. Lipid profile

Fatty acid methyl esters (FAMEs) were prepared from sea bass and sea bream head oils (500 µL) by transesterification with a methanol: sulfuric acid: toluene (2:1:1, *v/v/v*) catalytic solution (5 mL) overnight at 50 °C and 600 rpm. Then, 3 mL of water and 3 mL of diethyl ether were added and vortexed for 30 s in order to achieve phase differentiation. FAMEs were recovered from the upper layer and mixed with sodium sulfate. After filtering (0.22 µm nylon filters), samples were diluted 1/10 in diethyl ether and stored at – 20 °C until fatty acid analysis.

Fatty acid composition analysis was carried out using a gas chromatograph (GC) constituted by a DANI model GC 1000 instrument (Milan, Italy), a flame ionization detector (FID), a split/splitless injector, and a Macherey-Nagel capillary column (30 m × 0.32 mm ID × 0.25 µm d_f). Fatty acids were then identified by comparing the relative retention times of FAME peaks from fish head oils with a reference standard FAME mixture. Details of chromatography separation and determinations were described by Reis et al. (2012).

2.5.2. Lipid quality indices

The atherogenicity index (AI), the thrombogenicity index (TI), and the hypocholesterolemic index (HI) of the fish head oils, linking fatty acid profile to cardiovascular risk, were calculated according to the following equations (Chen and Liu, 2020; Mierliță, 2018; Ulbricht and Southgate, 1991):

$$AI = \frac{C12:0 + (4 \times C14:0) + C16:0}{MUFA + PUFA} \quad (2)$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{(0.5 \times MUFA) + (0.5 \times PUFAn6) + (3 \times PUFAn3) + \frac{PUFAn3}{PUFAn6}} \quad (3)$$

$$HI = \frac{C18:1 + PUFA}{C12:0 + C14:0 + C16:0} \quad (4)$$

The omega-3/omega-6 ratio, which is related to a healthy diet, was calculated from the relative percentages of fatty acids (Food and Agriculture Organization of the United Nations, 2010).

2.6. Evaluation of bioactive properties of head oils

2.6.1. Antimicrobial activity

Inhibitory activity of the obtained fish head oils was tested against eight bacteria (*Enterobacter cloacae* (ATCC 49741), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica* serotype Enteritidis (ATCC 13076), *Yersinia enterocolitica* (ATCC 8610), *Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 19111), and *Staphylococcus aureus* (ATCC 11632)) and two fungal strains (*Aspergillus fumigatus* (ATCC 204305) and *Aspergillus brasiliensis* (ATCC 16404)). All commercial microorganisms related to food contamination were purchased from Frilabo (Porto, Portugal).

Gram-positive bacteria were incubated in fresh Blood Agar (7% sheep blood) and Gram-negative bacteria in Muller Hilton Agar at 37 °C for 24 h in order to maintain the exponential growth phase. Bacterial suspensions were prepared at 1.5×10^6 CFU/mL. The micromycetes were grown in Malt Agar plates at 25 °C for 72 h. After this culture period, the spores were recovered from the agar surface with sterile 0.85% saline containing 0.1% Tween 80 (*v/v*). Fungal spore suspensions were adjusted at 1.0×10^5 UFC/mL. Before antimicrobial analysis, fish head oil samples were dissolved 50% in MHB (0.5% Tween 80) and then final concentrations to be tested were prepared by serial dilutions (50–0.39%).

To assess the antibacterial potential, the minimum inhibitory and minimum bactericidal concentrations (MIC and MBC, respectively) were evaluated using the broth microdilution method and the rapid INT colorimetric assay described by Pires et al. (2018). Ampicillin (20 µg/mL) and streptomycin (1 mg/mL) were used as positive controls for all bacteria, except for *S. aureus*, for which methicillin (1 mg/mL) was employed. Two negative controls (MHB and oil sample) were also prepared for each bacterial inoculum.

To determine the antifungal potential, the minimum inhibitory and minimum fungicidal concentrations (MIC and MFC, respectively) were evaluated according to the procedure reported by Heleno et al. (2013). Ketoconazole (1 mg/mL) was used as positive control while MHB and oil samples were utilized as a negative control for each fungal inoculum.

2.6.2. Antioxidant activity

The cellular antioxidant activity (CAA) assay described by Wolfe and Rui (2007) and adapted by de la Fuente et al. (2022) for salmon by-product oils was applied to evaluate the antioxidant capacity of sea bass and sea bream head oils. Murine macrophage cell line (RAW 264.7) was acquired from the European Collection of Authenticated Cell Cultures (ECACC). Cells were cultured in DMEM supplemented with 10% heat-inactivated FBS, L-glutamine (2 mM), penicillin (100 U/mL) and streptomycin (100 mg/mL) at 37 °C, 5% of CO_2 flow, and a humid atmosphere until 70–80% confluence of cell monolayer. Next, cells were scraped, seeded in 96-well plates at a density of 2×10^4 cells/well, and incubated for 48 h. Afterward, the culture medium was removed and the cells were washed twice with 100 µL of HBSS (100 mM, pH 7.4). Then, 200 µL of fish head oil at different concentrations (500–2000 µg/mL in DMSO:H_2O_2 1:1, *v/v*) and 100 µL of DCFH-DA (50 µM) were added to each well and co-incubated at 37 °C for 1 h. After incubation, the mixture was discarded and cells were washed twice with 100 µL of HBSS before adding 100 µL

of AAPH (600 μM). The reaction was carried out in a plate reader Biotek FLX800 (Bio-Tek Instruments, Inc., Winooski, VT, USA) with fluorescence filters for an excitation wavelength of 485 nm and an emission wavelength of 535 nm at 37 °C. The fluorescence values were recorded every 5 min over 1 h and the differences in areas under the curve (AUC) were considered for calculations. Therefore, CAA values were calculated according to Eq. (5), where ∫ AUCs is the integrated area under the sample fluorescence *versus* time curve, and ∫ AUCc is the integrated area from the control curve.

$$\text{CAA unit} = 100 - (\int \text{AUCs} / \int \text{AUCc}) \times 100 \quad (5)$$

The results were expressed as a percentage of inhibition of the oxidation reaction. Quercetin standard was used as a positive control.

2.6.3. Anti-inflammatory activity

The procedure reported by Sobral et al. (2016) to measure the inhibition of nitric oxide (NO) produced by LPS-stimulated macrophages was reproduced to evaluate the anti-inflammatory potential of sea bass and sea bream head oils. The quantification of NO was performed using a Griess Reagent System kit (Promega, Madison, WI, USA). For this method, RAW 264.7 cells were grown and maintained under the same culture conditions as for the aforementioned CAA assay. Then, they were seeded at 1.5×10^5 cells/well. Fish head oils were firstly dissolved in DMSO:H_2O (1:1, *v/v*) at a concentration of 8 mg/mL. Through serial dilutions, the final concentrations of fish oils tested were 6.25–400 μg/mL. Dexamethasone standard at 50 μM was used as positive control and head oil samples without LPS were employed as negative controls. Results were expressed as the oil concentration that caused 50% of NO production inhibition (IC_{50}, μg/mL).

2.6.4. Cytotoxic activity

Four human tumor cell lines (gastric adenocarcinoma, AGS; colon adenocarcinoma, CaCo-2; breast adenocarcinoma, MCF-7; and non-small cell lung cancer, NCI-H460) were used to evaluate the cytotoxic potential of sea bass and sea bream head oils. AGS and Caco-2 cells were provided by ECACC while MCF-7 and NCI-H460 cells were purchased from Leibniz-Institute DSMZ. All human tumor cell lines were grown and maintained in RPMI 1640 supplemented with 10% heat-inactivated FBS, L-glutamine (2 mM), penicillin (100 U/mL) and streptomycin (100 mg/mL). They were incubated at 37 °C with 5% CO_2 and humid atmosphere until 70–80% confluence of cell monolayer. All cancer cells were trypsinized and seeded at a density of 10,000 cells/well. In addition, one non-tumor cell line obtained from the primary culture of porcine liver (PLP2) was established in the laboratory and maintained according to the authors (Mandim et al., 2022). PLP2 cells were employed to verify that oil samples only affected cancer cells. On the other hand, the fish head oils were prepared at a final concentrations ranging from 6.25 to 400 μg/mL in DMSO:H_2O (1:1, *v/v*) (Kostić et al., 2022).

The sulforhodamine B (SRB) colorimetric assay (Sigma-Aldrich, St. Louis, MO, USA) described by Vichai and Kirtikara (2006) and adapted by de la Fuente et al. (2022) for salmon by-product oils was applied. Briefly, 100 μL of cold 10% (*w/v*) TCA were added to the wells and the microplates were incubated at 4 °C for 1 h. After removing the TCA, adhered cells were washed three times with water and were dried. Cell staining was then carried out by adding 100 μL of 0.057% (*w/v*) SRB solution at room temperature for 30 min. Excess dye was eliminated by washing three times with 1% (*v/v*) acetic acid and plates were left to dry. Next, 200 μL of 10 mM Tris base were used to dissolve the cells and the absorbance of protein-bound dye was measured in a microplate reader (Biotek ELX800, Bio-Tek Instruments, Inc., Winooski, VT, USA) at 510 nm. The antitumor drug ellipticine at 10 mM was used as a positive control. Plated cells without fish head oil were used as a negative control and their absorbance values were considered time zero for the calculations. Results were expressed as extract concentration responsible for 50% of cell growth inhibition (GI_{50}, μg/ mL).

2.7. Statistical analysis

Experimental data were subjected to one-way analysis of variance (ANOVA) to determine the significant differences among samples. Tukey Honestly Significant Difference (HSD) multiple range test ($p < 0.05$) was applied. The Statgraphics Centurion XVI® software (Statpoint Technologies, Inc., The Plains, VA, USA) was used for statistical analysis.

3. Results and discussion

3.1. Fish head oil extraction yields

The oil yield results of sea bass and sea bream head oils obtained by SE and MAE are shown in Table 1. The MAE technique resulted in extraction yields of 21.50 ± 1.85% and 20.75 ± 1.17% for sea bass and sea bream heads, respectively. Regarding SE, oil yields were 39.14 ± 0.65% for sea bass heads and 41.58 ± 0.67% for gilthead sea bream heads. Since Soxhlet method is supposed to extract total fat content from food samples, the extraction conditions of MAE allowed recovering 55% of total lipid content in sea bass heads and 52% in sea bream heads in less than 11 min. This means that MAE took about 33 times less extraction time (360 min *vs* 10.8 min) and 5 times less solvent amount (250 mL *vs* 50 mL) than SE, which is in accordance with the advantages related to the non-conventional MAE technique (Llompart et al., 2018).

On the other hand, a greater amount of fish oil was extracted by the traditional Soxhlet method than MAE process for both fish head samples. Recently, MAE has been used to optimize the oil recovery from salmon side streams, including heads (de la Fuente et al., 2022). Despite using the same extraction conditions, higher oil yield (38%) was obtained for salmon heads compared to sea bass and sea bream heads (21%). According to the data reported on the chemical composition of fish processing by-products, salmon heads contain a higher total lipid fraction than sea bass and sea bream heads (He et al., 2011; Valcarcel et al., 2020). Therefore, this difference could be due to the specific fish species employed for oil extraction, which is agreement with the conclusions reached by Ozogul et al. (2018). In addition, some studies have been carried out to compare the oil yield of fish fillets using traditional extraction methods and MAE technique (Costa and Bragagnolo, 2017; Ozogul et al., 2018; Ramalhosa et al., 2012). Different results were obtained in terms of oil yield after applying the different extraction

Table 1 – Oil yield and fatty acid profile of fish oil extracted from sea bass and gilthead sea bream heads using Soxhlet extraction (SE) and microwave-assisted extraction (MAE).

	Sea bass head oil		Sea bream head oil	
	SE	MAE	SE	MAE
Oil yield (%)				
g oil/100 g dw	39.14 ± 0.65^{b}	21.50 ± 1.85^{a}	41.58 ± 0.67^{b}	20.75 ± 1.17^{a}
Fatty acid profile (%)				
C12:0	0.03 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}
C14:0	2.22 ± 0.01^{a}	2.26 ± 0.01^{a}	2.67 ± 0.01^{b}	2.77 ± 0.05^{b}
C15:0	0.24 ± 0.01^{a}	0.22 ± 0.01^{a}	0.31 ± 0.01^{b}	0.32 ± 0.01^{b}
C16:0	14.72 ± 0.07^{a}	15.01 ± 0.06^{a}	14.37 ± 0.11^{a}	14.52 ± 0.16^{a}
C16:1	3.75 ± 0.04^{a}	3.80 ± 0.01^{a}	5.03 ± 0.06^{b}	5.14 ± 0.04^{b}
C17:0	0.18 ± 0.01^{a}	0.19 ± 0.01^{b}	0.23 ± 0.01^{c}	0.25 ± 0.01^{d}
C17:1	0.17 ± 0.01^{a}	0.16 ± 0.01^{a}	0.26 ± 0.02^{b}	0.29 ± 0.01^{b}
C18:0	2.73 ± 0.08^{a}	2.75 ± 0.14^{a}	2.69 ± 0.02^{a}	2.77 ± 0.01^{a}
C18:1n9c	33.73 ± 0.18^{a}	34.41 ± 0.41^{a}	35.30 ± 0.13^{b}	36.64 ± 0.41^{b}
C18:2n6t	0.44 ± 0.13^{a}	0.33 ± 0.01^{a}	nd	nd
C18:2n6c	16.29 ± 0.32^{b}	16.52 ± 0.13^{b}	12.90 ± 0.07^{a}	13.06 ± 0.19^{a}
C18:3n6	0.32 ± 0.05^{b}	0.26 ± 0.01^{b}	0.20 ± 0.01^{a}	0.19 ± 0.01^{a}
C18:3n3	3.73 ± 0.02^{b}	3.60 ± 0.11^{b}	3.03 ± 0.01^{a}	3.00 ± 0.01^{a}
C20:0	0.08 ± 0.01^{a}	0.07 ± 0.01^{a}	0.13 ± 0.01^{b}	0.12 ± 0.01^{b}
C20:1	1.91 ± 0.05^{b}	1.88 ± 0.02^{b}	1.50 ± 0.01^{a}	1.56 ± 0.02^{a}
C20:2	0.95 ± 0.02^{b}	0.95 ± 0.02^{b}	0.39 ± 0.01^{a}	0.41 ± 0.01^{a}
C20:3n6	0.21 ± 0.01^{a}	0.20 ± 0.01^{a}	0.19 ± 0.01^{a}	0.19 ± 0.01^{a}
C20:4n6	0.47 ± 0.02^{ab}	0.44 ± 0.01^{a}	0.52 ± 0.01^{b}	0.48 ± 0.02^{ab}
C20:3n3	0.26 ± 0.01^{c}	0.26 ± 0.01^{c}	0.19 ± 0.01^{a}	0.21 ± 0.01^{b}
C22:0	0.05 ± 0.01^{a}	0.08 ± 0.01^{a}	0.12 ± 0.01^{b}	0.14 ± 0.01^{b}
C20:5n3	4.60 ± 0.13^{a}	4.37 ± 0.20^{a}	4.33 ± 0.14^{a}	4.41 ± 0.16^{a}
C22:2	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}
C24:1	1.33 ± 0.03^{a}	1.10 ± 0.01^{a}	1.94 ± 0.04^{a}	1.64 ± 0.12^{a}
C22:6n3	11.54 ± 0.18^{ab}	11.02 ± 0.10^{a}	13.61 ± 0.17^{c}	12.79 ± 0.21^{b}
Total fatty acid class				
SFA	20.25 ± 0.02^{a}	20.68 ± 0.12^{ab}	20.57 ± 0.08^{ab}	20.94 ± 0.25^{b}
MUFA	40.89 ± 0.23^{a}	41.34 ± 0.38^{a}	44.03 ± 0.16^{b}	45.27 ± 0.59^{b}
PUFA	37.87 ± 0.22^{b}	36.98 ± 0.29^{b}	35.35 ± 0.25^{a}	34.73 ± 0.57^{a}
Oil quality indices				
n6/n3	0.88 ± 0.01^{b}	0.92 ± 0.02^{c}	0.65 ± 0.01^{a}	0.68 ± 0.01^{a}
AI	0.30 ± 0.01^{a}	0.31 ± 0.02^{a}	0.32 ± 0.01^{b}	0.32 ± 0.01^{b}
TI	0.15 ± 0.01^{b}	0.15 ± 0.01^{b}	0.11 ± 0.01^{a}	0.12 ± 0.01^{a}
HI	4.22 ± 0.01^{b}	4.11 ± 0.01^{a}	4.14 ± 0.03^{a}	4.12 ± 0.01^{a}

C12:0 lauric acid; C14:0 mystiric acid; C15:0 pentadecylic acid; C16:0 palmitic acid, C16:1 palmitoleic acid; C17:0 heptadecanoic acid; C17:1 heptadecenoic acid; C18:0 stearic acid; C18:1n9c oleic acid; C18:2n6t linolelaidic acid C18:2n6c linoleic acid; C18:3n6 γ-linoleic acid; C18:3n3 α-linolenic acid; C20:0 arachidic acid; C20:1 eicosenoic acid; C20:2 eicosadienoic acid; C20:3n6 eicosadienoic acid; C20:4n6 arachidonic acid; C20:3n3 eicosatrienoic acid; C22:0 docosanoic acid; C20:5n3 eicosapentaenoic acid (EPA); C22:2 docosadienoic acid; C24:1 nervonic acid; C22:6n3 docosahexaenoic acid (DHA); nd: not detected; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n6/n3: omega-6/omega-3 ratio; AI: atherogenicity index; TI: thrombogenicity index; HI: hypocholesterolemic index. In each row, different letters mean statistical differences ($p < 0.05$) among fish oil samples.

processes. As a consequence, further research is required, especially for fish processing side streams.

3.2. *Nutritional properties of fish head oils*

3.2.1. *Lipid profile*

Fatty acid composition of sea bass and sea bream head oils recovery by SE and MAE are listed in Table 1. The results showed that the extraction method did not affect the fatty acid composition of head oils. In addition, few variations considering the lipid profile were observed among fish species. The predominant fatty acid identified in both oil samples was oleic acid (C18:1n9c), with a slight difference ($p < 0.05$) between sea bass (33–34%) and sea bream (35–36%). Linoleic acid (C18:2n6c, 13–16%), palmitic acid (C16:0, 14–15%), docosahexaenoic acid (DHA, 11–14%), eicosapentaenoic acid (EPA, ≈4%), and linolenic acid (C18:3n3, 3–4%) were then the most representative fatty acids for both fish head oils. Total saturated fatty acids (SFA, 20–21%) were equivalent between oil samples. However, some differences ($p < 0.05$) in the percentages of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were found. Thus, oil from sea bass heads showed ≈ 41% MUFA and ≈ 37% PUFA while oil from sea bream heads presented ≈ 44% MUFA and ≈ 35% PUFA.

Similar percentages of oleic, linoleic, and palmitic acids were found in head oils of European sea bass (Munekata et al., 2020) and gilthead sea bream (Pateiro et al., 2020). Few differences were also observed between MUFA and PUFA content, which seemed to be linked to the greater amount of EPA and mainly DHA in the fish head samples analyzed in the present study. Interestingly, the levels of DHA were higher than those found in heads from the same fish species (Munekata et al., 2020; Pateiro et al., 2020), as well as equivalent to or higher than those reported for salmon heads (de la Fuente et al., 2022; He et al., 2011; Inguglia et al., 2020).

"High omega-3 fatty acids" is a food claim established by European authorities when a food product contains at least 80 mg of DHA+EPA per 100 g and per 100 Kcal (Regulation (EU) No 1924/2006). Since sea bass and sea bream head oils here investigated presented an average of 15.7 and 17.6 g of DHA+EPA/100 g, respectively, both fish oils comply with this health claim.

3.2.2. Fish quality indices

Fish quality indices of sea bass and sea bream head oils recovery by SE and MAE are reported in Table 1. All oils presented AI values below 0.35, indicating the low atherogenic potential of the fatty acids of these fish head oils. This is due to the presence of a greater amount of fatty acids considered anti-atherogenic (MUFAs and PUFAs) compared to fatty acids considered pro-atherogenic (C12:0, C14:0, and C16:0) (Chen and Liu, 2020; Mierliță, 2018). AI values (0.21–1.86) of several fish species have been previously reported (Chen and Liu, 2020; Moussa et al., 2014). Similarly, TI values below 0.15 for all oils studied also show low thrombogenic potential due to the higher proportion of anti-thrombogenic fatty acids (MUFAs and the n3 and n6 families) relative to pro-thrombogenic fatty acids (C12:0, C14:0, and C16:0) (Chen and Liu, 2020; Mierliță, 2018). TI values (0.14–0.87) of various fish species have been also reviewed (Chen and Liu, 2020). Not only AI and TI but also HI assess the potential effect of fatty acid composition on cardiovascular health. The results of HI ranged from 4.11 to 4.22 for all fish head oils, with values equal to or higher than those obtained for different species of fish, shellfish, and algae (0.21–4.22), except in the case of the decapod crustacean *Cancer edwardsi* (4.75) (Chen and Liu, 2020). Since a high HI together with low AI and TI are considered to contribute to a decrease in cardiovascular risk, oils from sea bass and sea bream heads have high-quality nutritional properties. Furthermore, omega-6/omega-3 lower than 4 is related to a healthy diet (FAO, 2010). The omega-6/omega-3 ratios of fish head oil samples were 0.88–0.92 (sea bass) and 0.64–0.68 (sea bream), thus meeting this quality criterion.

According to the nutritional attributes exhibited by sea bass and sea bream head oils, they could be part of the human diet. In addition, these underutilized fish processing by-products are interesting candidates for the production of oil intended for feed and food fortification in order to achieve a better lipid profile of the final product. In this sense, meat, milk, bakery products, and livestock feed are currently fortified using fish oil (Jamshidi et al., 2020).

3.3. Bioactivities of sea bass and gilthead sea bream head oils

3.3.1. Antimicrobial activity

Results of antibacterial and antifungal activities of sea bass and sea bream head oils extracted by SE and MAE are presented in Table 2. All fish oils inhibited the growth of all bacteria and fungi tested. It should be highlighted that the head oils obtained by MAE exhibited a higher antibacterial effect than the oils extracted by Soxhlet, except for *P. aeruginosa*, against which there were no differences between extraction methods. In addition, sea bream head oil was more effective against *E. cloacae*, *S. enterica*, and *S. aureus* than sea bass head oil. In the same way, salmon head oil inhibited the growth of *P. aeruginosa* and *S. aureus* (Inguglia et al., 2020). Recently, the antibacterial potential of cephalopod liver viscera oil against several clinical bacteria has also been reported (Moovendhan et al., 2021). Regarding antifungal activity, equivalent results were observed for sea bass and sea bream head oils against *A. brasiliensis* and *A. fumigatus*. Similar results were found for salmon by-product oils against *A. brasiliensis* (de la Fuente et al., 2022). Neither sea bass nor sea bream head oil showed bactericidal or fungicidal activity against the microorganisms tested, while salmon backbones and head oils did exhibit fungicidal effects on *A. fumigatus* (de la Fuente et al., 2022).

Because there are no differences in the fatty acid composition of the head oils concerning the extraction techniques (Table 1), other constituents present in these oils could influence the antimicrobial activity. Although the literature information on the content of fat-soluble vitamins in fish by-

Table 2 – Antibacterial and antifungal activities of fish oil extracted from sea bass and sea bream heads using Soxhlet extraction (SE) and microwave-assisted extraction (MAE).

	Sea bass head oil				Sea bream head oil			
	SE		MAE		SE		MAE	
Antibacterial activity	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria								
E. cloacae	50	na	12.5	na	50	na	3.125	na
E. coli	25	na	25	na	25	na	12.5	na
P. aeruginosa	50	na	50	na	50	na	50	na
S. enterocolitica	25	na	25	na	25	na	6.25	na
Y. enterocolitica	12.5	na	3.125	na	12.5	na	3.125	na
Gram-positive bacteria								
B. cereus	25	na	12.5	na	25	na	12.5	na
L. monocytogenes	50	na	50	na	25	na	6.25	na
S. aureus	12.5	na	6.25	na	25	na	3.125	na
Antifungal activity	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
A. brasiliensis	50	na	25	na	25	na	25	na
A. fumigatus	50	na	25	na	50	na	25	na

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; MFC: minimum fungicidal concentration; na: no activity; MIC and MBC values for positive controls: streptomycin (0.007/0.01 mg/mL), methicillin (0.007/0.007 mg/mL), ampicillin (0.15/0.63 mg/mL), and ketoconazole (0.06/0.5 mg/mL); MFC values for positive controls: ketoconazole (0.125/1.0 mg/mL).

Table 3 – Antioxidant, anti-inflammatory, and cytotoxic activities of fish oil extracted from sea bass and sea bream heads using Soxhlet extraction (SE) and microwave-assisted extraction (MAE).

	Sea bass head oil		Sea bream head oil	
	SE	MAE	SE	MAE
Cellular antioxidant activity (oxidation inhibition % at 2 mg/mL)				
RAW 264.7	56.00 ± 5.74[b]	35.00 ± 4.45[a]	na	29.00 ± 3.20[a]
Anti-inflammatory activity (IC_{50} µg/mL)				
RAW 264.7	14.94 ± 0.14[a]	20.84 ± 1.30[b]	14.84 ± 0.70[a]	13.89 ± 1.28[a]
Cytotoxic activity (GI_{50} µg/mL)				
AGS	240.39 ± 10.89[a]	250.21 ± 10.77[a]	249.12 ± 16.31[a]	273.94 ± 8.95[a]
Caco-2	> 400 b	224.00 ± 12.63[a]	361.76 ± 33.97[a]	325.73 ± 21.49[a]
MCF-7	93.07 ± 0.99[b]	38.17 ± 2.67[a]	39.43 ± 0.92[a]	46.23 ± 4.65[a]
NCI-H460	144.18 ± 14.19[a]	235.70 ± 23.28[b]	233.63 ± 3.53[b]	178.67 ± 2.55[ab]
PLP2	227.65 ± 6.24[b]	236.73 ± 17.54[b]	237.68 ± 12.96[b]	156.50 ± 10.72[a]

IC50 values for ellipticine: 1.23 ± 0.03 µg/mL (AGS), 1.21 ± 0.02 µg/mL (Caco-2), 1.02 ± 0.02 µg/mL (MCF-7), 1.02 ± 0.01 µg/mL (NCI-H460), 1.4 ± 0.1 µg/mL (PLP2). IC50 values for dexamethasone: 6.3 ± 0.4 µg/mL (RAW 264.7). Quercetin: 95 ± 5% oxidation inhibition at 0.3 µg/mL; na: no activity. Different letters in each row correspond to significant differences ($p < 0.05$) among oil samples.

products is scarce, fish liver oils from tuna, shark, and cod are considered important sources of vitamins A and E (Moovendhan et al., 2021). Since vitamins are known to be heat sensitive, the temperatures reached in the Soxhlet extraction process (≈80 °C) are likely to degrade the vitamins present in fish head oils. In contrast, the MAE technique, applied here at a maximum temperature of 50 °C, could have prevented the degradation of vitamins in the obtained oils. Therefore, sea bass and sea bream head oils extracted by MAE could exert antibacterial activity due to the content of vitamins or other thermolabile compounds. Further research is required in this regard.

3.3.2. *Cellular antioxidant activity*

Results of antioxidant activity of sea bass and sea bream head oils extracted by SE and MAE are shown in Table 3. The highest oil concentration tested (2 mg/mL) inhibited the oxidation reaction generated in the RAW macrophages by 29–56%, except for the sea bream head oil extracted by Soxhlet, which did not show antioxidant capacity. In a previous study, head oil from Atlantic salmon (*Salmo salar*) obtained by MAE also inhibited the oxidation reaction by 36% while that extracted by Soxhlet did not exhibit antioxidant potential (de la Fuente et al., 2022). The traditional Folch method was used to recover oil from hake (*Merluccius merluccius*) heads and the results revealed high antioxidant activity based on DPPH radical scavenging activity and β-carotene bleaching inhibition assays (Karoud et al., 2020).

3.3.3. *Anti-inflammatory activity*

Results of anti-inflammatory activity of sea bass and sea bream head oils extracted by SE and MAE are shown in Table 3. Regardless of the extraction method used, all fish head oils tested exhibited important NO inhibition in LPS-stimulated RAW macrophage cells. The concentration of fish head oils required to inhibit 50% NO production was from 14 to 21 µg/mL. Thus, the anti-inflammatory potential of sea bass and sea bream head oils are higher than that of salmon heads (51–75 µg/mL), backbones (34–63 µg/mL), and viscera (76–79 µg/mL) also obtained by Soxhlet method and MAE technique (de la Fuente et al., 2022). In addition, the administration of oil from the hake head reduced significantly the edema in a carrageenan-induced mice paw edema model (Karoud et al., 2020). The authors suggested that the anti-inflammatory effect of hake head oil could be due to the presence of EPA and DHA, since these fatty acids act as competitor substrates for the inhibition oxidation of arachidonic acid. In the same way, high levels of EPA and DHA as well as low levels of MUFA from marine sources and related by-products were associated with the inhibition of NO and TNFα in LPS-stimulated macrophage cells (Ahmad et al., 2019). Therefore, the anti-inflammatory activity showed by sea bass and sea bream head oils could be due to their relevant content of EPA and, especially, DHA (Table 1). Addressing the cellular mechanisms involved in the observed anti-inflammatory effect is an important line of research for future work.

3.3.4. *Cytotoxic activity*

Data regarding cytotoxicity of sea bass and sea bream head oils obtained by SE and MAE are presented in Table 3. The inhibition of cancer cell growth by the fish head oils tested did not seem to be related to the oil extraction techniques used. The breast cancer cells (MFC-7) exhibited the highest susceptibility to the oil samples (GI_{50} = 38–93 µg/mL) while the colon cancer cells (Caco-2) showed the lowest, at the tested concentrations. Similar growth inhibition effects of stomach (AGS) and lung cancer (NCI-H460) cell lines were observed for all fish head oils. Salmon head oil obtained by MAE technique also inhibited proliferation of MFC-7 cells (GI_{50} = 131 µg/mL) (de la Fuente et al., 2022). According to the literature, cytotoxic or antiproliferative *in vitro* studies have been performed using isolated fish oil fatty acids instead of oil from fish or fish processing by-products. As a result of the data reviewed by Jameel et al. (2019), different omega-3 fatty acids from fish oil could be considered anti-cancer agents since they influence multiple mechanisms involved in cancer development. As for the omega-3 fatty acid DHA, found in high levels in the studied fish head oil samples, some works have revealed its antiproliferative effect against colon and lung cancer cells (Ahangar et al., 2016; Yin et al., 2017).

4. Conclusions

The MAE technique recovered more than 50% of oil from sea bass and sea bream heads in less than 11 min. The fatty acid composition of the oils showed high levels of DHA and a potential protective effect on cardiovascular risk. There was

a remarkable inhibition of bacterial growth, which could be due to non-degraded thermolabile compounds during oil extraction by MAE. Fish heads oils also exhibited a relevant anti-inflammatory effect and great cytotoxic potential against breast cancer cells. Overall, this work represents a first step towards the valorization of sea bass and sea bream heads under a circular economy approach.

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.

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