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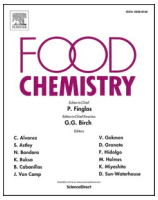

Nutritional and bioactive oils from salmon (*Salmo salar*) side streams obtained by Soxhlet and optimized microwave-assisted extraction

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ABSTRACT

The efficiency of the microwave-assisted extraction (MAE) technique on recovering nutritional and bioactive oils from salmon (*Salmo salar*) side streams was evaluated and compared to Soxhlet extraction. The response surface methodology (RSM) coupled with a central composite rotatable design was used to optimize time, microwave power, and solid/liquid ratio of the MAE process in terms of oil yield. The optimal MAE conditions were 14.6 min, 291.9 W, 80.1 g/L for backbones, 10.8 min, 50.0 W, 80.0 g/L for heads, and 14.3 min, 960.6 W, 99.5 g/L for viscera, which resulted in a recovery of 69% of the total lipid content for backbones and heads and 92% for viscera. The oils obtained under optimal MAE conditions showed a healthy lipid profile as well as cytotoxic, antioxidant, anti-inflammatory, or antimicrobial properties. These results highlight that oils from underutilized salmon by-products could be exploited by different industrial sectors under the circular economy approach.

1. Introduction

The concept of circular economy applied to the food industry has been promoted by the European Union in recent years. The transformation of food bio-waste into value-added products is now a challenge for both the food industry and the researchers. This approach is particularly interesting for the seafood processing industry since it can generate up to 80% by-products from the whole organism (Nawaz et al., 2020). In this context, several studies have highlighted the nutritional value and functional properties of fish processing side streams, showing their relevance from a food perspective (Al Khawli, Pateiro, Domínguez, Lorenzo, Gullón, Kousoulaki, & Barba, 2019; Nawaz et al., 2020). A case study of salmon aquaculture concluded that there are economic, environmental, and food security benefits through an appropriated management of salmon by-products, which were also considered food grade raw material for human consumption (Stevens, Newton, Tlusty, & Little, 2018).

Atlantic salmon (*Salmo salar*) is currently the most important farmed fish in Europe, reaching 1.3 million tons and 5 billion EUR in 2017 (European Commision, 2020). About 50% of a whole salmon is transformed into marketable edible products while the remaining 50% (mainly backbones, heads, and viscera) is available for the recovery of quality nutrients and bioactive compounds (He, Franco, & Zhang, 2011; Inguglia et al., 2020). In this sense, salmon side streams are considered to be rich in fat (15–30%) and a good source of polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (He et al., 2011).

EPA and DHA are recognized to be the most important fatty acids of the omega-3 PUFAs family due to their proven and potential biological activities. These fish fatty acids are accepted to prevent risk factors associated with the development of various disorders such as cardiovascular and neurological diseases, inflammation, hypertension, obesity, osteoarthritis, and different types of cancer (Kapoor, Kapoor, Gautam, Singh, & Bhardwaj, 2021; Oppedisano, Macrì, Gliozzi, Musolino, Carresi, Maiuolo, & Mollace, 2020). A great number of studies have also shown cytotoxic or anti-proliferative effects of omega-3 PUFAs from fish on different human cancer cell lines without affecting normal cells (Jameel, Agarwal, Arshad, & Serajuddin, 2019). In addition, the antibacterial activity related to specific compounds such as EPA, DHA, and linolenic acid have been described (Inguglia et al., 2020).

Dietary foods containing PUFAs have been recommended to include in a routine diet in order to maintain a good health (Kapoor et al., 2021).

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Fish oil has been widely applied for the fortification of different food products, an innovative strategy accepted by consumers interested in healthy foodstuffs (Jamshidi, Cao, Xiao, & Simal-Gandara, 2020). The growing demand for fish oil has led to the use of fish processing by-products (mainly cod liver) for oil production by the industry (Liu, Ramakrishnan, & Dave, 2020). At industrial scale, wet pressing is the conventional technique globally applied to obtain fish oil. However, this process involves two important disadvantages, an inefficient oil extraction and the degradation of thermolabile compounds. Due to moderate extraction conditions, enzymatic extraction became an initial alternative for the extraction of oil from fish side streams. Nevertheless, the oil yield was not as efficient as expected (Bruno, Ekorong, Karkal, Cathrine, & Kudre, 2019). Recently, non-conventional extraction techniques such as supercritical fluid extraction, ultrasound-assisted extraction, and microwave-assisted extraction (MAE) have been proposed as potential sustainable methods for oil extraction from fish by-products (Al Khawli et al., 2019; Alfio, Manzo, & Micillo, 2021; Bruno et al., 2019; Marsol-Vall, Aitta, Guo, & Yang, 2020).

MAE is based on the use of microwave energy to rapidly heat solid samples in contact with solvents to improve the extraction of compounds (Llompart, Garcia-Jares, Celeiro, & Dagnac, 2018). The increase in temperature causes an increase in pressure in the cells of the sample until a massive disruption of cell membranes, facilitating the transfer of compounds to the solvent (Alfio et al., 2021; Marsol-Vall et al., 2020; Ozogul et al., 2018). As a modern technique, MAE is also appreciated for the possibility of automating the extraction process as well as for using short extraction times and few amount of solvent. A greater extraction yields of bioactive compounds from plant matrices by MAE have been reported (Bagade & Patil, 2019). However, studies related to MAE technique for the extraction of fish oils are scare. For instance, MAE was used to recover oil from edible muscles of different fish species and authors observed different effects of MAE on oil yield and quality (Afolabi, Mudalip, & Alara, 2018; dos Costa & Bragagnolo, 2017; Ozogul et al., 2018). As far as we know, only one study about MAE and fish by-products oil has been published. In that work, MAE was employed effectively as a pre-treatment for the enzymatic extraction of head oil from the freshwater fish *Labeo rohita* (Bruno et al., 2019). Therefore, there is currently a research gap in this field and further research is required.

The main goal of the present study was to explore, for the first time, the application of MAE technique to recover fish oil enriched in omega-3 PUFAs from salmon side streams. Backbones, heads, and viscera were used to optimize the MAE of oils applying the response surface methodology (RSM). Oil yield as well as nutritional (fatty acid profile) and bioactive (cytotoxic, antioxidant, anti-inflammatory, and antimicrobial) properties of salmon oils obtained under MAE optimal conditions were evaluated and compared with those of the traditional Soxhlet extraction.

2. Material and methods

2.1. Standards and reagents

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

2.2. Fish material and sample preparation

Salmon backbones, heads, and viscera were provided by the Department of Nutrition and Feed Technology of the Nofima Food, Fisheries and Aquaculture Research Institute (Bergen, Norway). The salmon side stream materials were collected fresh from the filleting factory at Sotra Seafood (Oygaraden, Norway), stored frozen (-80 °C for 48 h) and freeze-dried (-56 °C for 72 h). Lyophilized samples were then vacuum packed and transported under refrigeration conditions to the Mountain Research Center (CIMO, Bragança, Portugal). Each type of salmon side stream was minced in order to obtain a smaller particle size for a better sample homogenization. Fish samples were stored at −25 °C until use.

2.3. Experimental design for MAE optimization

A central composite rotatable design (CCRD) combining five-levels of the independent variables X_1 (time, *t*, 1–20 min), X_2 (microwave power, *P*, 50–1000 W), and X_3 (solid/liquid ratio, *R*, 80–120 g/L) was implemented to optimize the extraction of fatty acids from salmon by-products using RSM. These variables and the respective range of values were selected based on preliminary experiments and literature data (Afolabi et al., 2018; dos Costa & Bragagnolo, 2017; Ozogul et al., 2018). Design-Expert software, Version 11 (Stat-Ease, Inc., Minneapolis, MN, USA) was used to generate the experimental points of the CCRD design, which included eight factorial points, six axial or star points, and six replicated center points. The 20 runs were randomized to minimize the effects of unexpected variability.

2.4. Extraction methods

2.4.1. Soxhlet extraction (SE)

A conventional SE was performed using 5 g of fish sample and ~ 250 mL of *n*-hexane in a laboratory Soxhlet extractor (Behr Labor Technik™, Düsseldorf, Germany) at 80 °C for 6 h. Following the extraction process, the solvent was completely removed using a rotary vacuum evaporator (Hei-VAP Silver 4, Schwabach, Germany) with a water bath at 40 °C. All extractions were carried out at least in duplicate.

2.4.2. Microwave-assisted extraction (MAE)

The MAE process was conducted in a Nμ Tech microwave extractor (NuWav-Uno, Sonilex, West Bengal, India) equipped with a circulating cool-water reflux system, time controller, and manual electromagnetic stirrer. The Intelli-System's internal also controls the microwave power conditions (max. 1000 W) thus eliminating any temperature overshoot. The quantities of fish sample (backbones, heads or viscera) and solvent (*n*-hexane) required to obtain the designed solid–liquid ratio with a constant volume of 50 mL were introduced into a flask, which was placed in the microwave chamber. For each extraction (performed according to the CCRD), time and power were set by the digital panel. After extraction, samples were filtered and the solvent was removed as described for SE.

2.5. Oil yield determination

For both extraction methods, the resulting amount of oil from salmon side streams was calculated gravimetrically as follows: extraction yield of oil (%) = (weight of extracted oil / weight of fish material) × 100. Since lyophilized fish samples were used, percent recovery values refer to dry weight (dw).

2.6. Characterization of the fatty acid profile

The fatty acids of salmon oils extracted by SE and MAE were subjected to methyl esterification according to Reis, Barros, Martins, and Ferreira (2012) with some modifications. Thus, 5 mL of a catalytic solution of methanol:sulfuric acid:toluene (2:1:1, *v/v/v*) were mixed with 500 μL of oil sample and kept overnight in a bath at 50 °C and 60 rpm. Then, 3 mL of water and 3 mL of diethyl ether were added and vortexed for 30 s. After phase differentiation, the obtained FAMEs were recovered from the upper layer, mixed with sodium sulfate and filtered (0.22 μm nylon filters). All samples were diluted 1/10 in ethyl ether before being stored at −20 °C until analysis.

Fatty acid profile analysis was performed using a gas–liquid chromatography with flame ionization detection (GC-FID). A DANI model GC 1000 instrument (Milan, Italy) equipped with a split/splitless injector, a FID detector, and a Macherey-Nagel capillary column (30 m × 0.32 mm ID × 0.25 μm d_f) was employed for chromatographic determinations. Hydrogen at a flow rate of 4 mL/min was used as gas carrier. The injector and detector temperatures were 250 °C and 260 °C, respectively. The oven temperature program was as follows: 50 °C for 2 min, increased to 125 °C at 30 °C/min, increased to 160 °C at 20 °C/min, increased to 180 °C at 3 °C/min, increased to 200 °C at 20 °C/min and increased to 220 °C for 15 min. One microliter of sample was injected and the fatty acids identification was carried out by comparing the relative retention times of FAMEs peaks from salmon oils with a reference standard FAMEs mixture. For data recorded and processed, CSW 1.7 software (DataApex 1.7) was used. The results were expressed in relative percentage of each fatty acid.

2.7. Response variables, extraction process modelling and statistical analysis

The fatty acid profile of the lipid extracts obtained with the 20 runs of the experimental design was analyzed by GC-FID for each sample (results in Tables A.1–A.3 provided in Supplementary Material). Since fatty acids were not significantly affected by the applied MAE conditions, these were not used as response variable. Therefore, the oil yield (g/100 g dw) was the dependent variable selected to optimize the extraction process.

The response surface models were fitted by means of least squares calculation using the second-order polynomial Eq. (1):

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} {X_i}^2 + \sum_{\substack{i=1 \\ j>i}}^{n-1} \sum_{j=2}^{n} b_{ij} X_i X_j \quad (1)$$

where Y corresponds to the dependent variable to be modelled (oil yield), X_i and X_j define the independent variables, b_0 is the constant coefficient, b_i is the coefficient of the linear effect, b_{ii} is the coefficient of the quadratic effect, b_{ij} is the coefficient of the interaction effect, and n is the number of variables ($n = 3$).

Fitting procedures, coefficient estimates, and statistical analysis were performed using Design-Expert software. Analysis of variance (ANOVA) was used to assess the significance of the models and of all the terms that make up the models, as well as the lack-of-fit. Only the statistically significant terms ($p < 0.05$) were used in the models' construction (except those required to ensure hierarchy). Coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}), and adequate precision were used to estimate the adequacy of the polynomial equation to the response. Since the lack-of-fit measures the quality of the model's fit to the experimental data, it must be non-significant (p greater than 0.05).

2.8. Biological activities of salmon oils obtained under optimized conditions

2.8.1. Cell lines and culture conditions for cellular assays

Different cell lines were used to evaluate different *in vitro* bioactivities of salmon oils extracted by SE and optimized MAE: Human gastric (AGS), colorectal (CaCo-2), breast (MCF-7), and lung (NCI-H460) cancer cells; non-tumor porcine liver primary culture (PLP2) cells, as well as murine macrophage (RAW 264.7) cells. AGS, Caco-2 and RAW cells were purchased from the European Collection of Authenticated Cell Cultures (ECACC) while MCF-7 and NCI-H460 cells were provided by Leibniz-Institute DSMZ. In compliance with the authors (Mandim et al., 2022), the primary culture PLP2 was established in the laboratory using porcine liver tissue in order to obtain tissue explants for the proliferation of non-tumor liver cells.

Tumor and non-tumor cell lines were grown and maintained in RPMI 1640 while RAW cells did so in DMEM. Both culture mediums were supplemented with 10% heat-inactivated FBS, L-glutamine (2 mM), penicillin (100 U/mL) and streptomycin (100 mg/mL). All cell types were incubated in culture flasks (75 cm^2) at 37 °C, a CO_2 flow of 5% and under a humid atmosphere. The medium was changed every 2–3 days until the cell monolayer reached 70–80% confluence. Afterward, tumor and non-tumor cells were harvested by trypsinization whilst murine macrophages were scraped. Then, cells were seeded in 96-well plates at a density (cells/cm^2) depended on the cell type.

Tumor cells were employed for cytotoxic evaluation. PLP2 cells were used to verify that the effect of salmon oils only affected to tumor cells. RAW cells were used for antioxidant and anti-inflammatory tests. For all cellular assays, the incubation conditions were the same as for cell culture maintenance (37 °C/ 5% CO_2/ humid atmosphere). All assays were performed with at least two technical replicates in two independent days.

2.8.2. Fish oil sample preparation for cellular assays

Salmon backbones, heads, and viscera oils extracted by SE and optimized MAE were used for cell bioactivity studies. Each oil sample was previously dissolved in DMSO:H_2O (1:1, *v/v*) at a concentration of 8 mg/mL (stock solution). Serial dilutions were then prepared in a microplate at a concentration range of 0.125–8 mg/mL, before performing the specific cell assay. The final concentrations of salmon oils tested were 6.25–400 μg/mL for cytotoxic and anti-inflammatory cell assays, while 500–2000 μg/mL were used for the cellular antioxidant activity (CAA) assay.

2.8.3. Cytotoxic activity

Tumor and non-tumor cells mentioned above were used to evaluate the cytotoxic effect of salmon oils extracted by SE and MAE. All cell lines were seeded at a density of 10,000 cells/well. According to the procedure previously described by Mandim et al. (2022), 190 μL of cell suspension were added to 10 μL of different concentrations of oil solutions and kept for 40 min at room temperature. After verifying the correct cellular distribution and adherence in the wells, the plates were incubated at 37 °C for 72 h. Then, sulforhodamine B (SRB) colorimetric assay (Sigma-Aldrich, St. Louis, MO, USA) for the cytotoxicity screening of compounds to adherent cells was applied (Vichai & Kirtikara, 2006). For this, 100 μL of cold 10% (*w/v*) TCA were added to the wells and the plates were incubated at 4 °C for 1 h. Next, TCA was removed and adhered cells were washed three times with water and dried. Cells were then stained with 100 μL of 0.057% (w/v) SRB solution for 30 min at room temperature. Excess dye was removed by washing three times with

1% (*v/v*) acetic acid. Once the plates were dried, 200 μL of 10 mM Tris base were used to dissolve the cells and the absorbance at 510 nm of protein-bound dye was measured in a microplate reader (Biotek ELX800, Bio-Tek Instruments, Inc., Winooski, VT, USA). For each cell line, plated cells without salmon oil were used as a negative control and their absorbance values were considered time zero for the calculations. In addition, the antitumor drug ellipticine (10 mM) was used as a positive control. The results were expressed as GI_{50} values (oil concentration capable of inhibiting 50% of cancer cell growth).

2.8.4. Antioxidant activity

The evaluation of the antioxidant capacity of salmon oils was carried out using the cellular antioxidant activity (CAA) assay described by Wolfe and Rui (2007), with some modifications. This method is based on the determination of intracellular reactive oxygen and nitrogen species (ROS/RNS) by measuring the ability of compounds to prevent the oxidation of intracellular dihydrodichlorofluorescein ($DCFH_2$), which is easily oxidizable to fluorescent dichlorofluorescein (DCF) by peroxyl radicals ($ROO^{\bullet}$). The RAW 264.7 cells were seeded at a density of 2×10^4 cells/well and incubated for 48 h. After this period, the culture medium was discarded and the cells were washed twice with 100 μL of HBSS (100 mM, pH 7.4). Then, 200 μL of oil at different concentrations 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 mixtures were removed and cells were washed twice with 100 μL of HBSS. Next, 100 μL of AAPH (600 μM), an azo compound that generates $ROO^{\bullet}$ by thermal decomposition, were added to each well. The reaction was performed at 37 °C 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. The fluorescence was recorded every 5 min over 1 h and the differences of areas under the curve (AUC) were considered for calculations. Therefore, CAA values were calculated according to Eq. (2), where $\int$ AUCs is the integrated area under the sample fluorescence *versus* time curve and $\int$ AUCc is the integrated area from the control curve.

$$\text{CAA unit} = 100 - \left(\int \text{AUCs} / \int AUCc \right) \times 100 \quad (2)$$

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

2.8.5. Anti-inflammatory activity

The inhibition of nitric oxide (NO) produced by LPS-stimulated RAW 264.7 cells was determined according to Sobral et al. (2016), with some modifications. RAW cells were seeded at 1.5×10^5 cells/well and growth overnight under incubation conditions in order to attach to the plate. Then, cells were exposed to the different concentrations of each fish oil (15 μL) and they were incubated for 1 h. The anti-inflammatory corticosteroid dexamethasone (50 mM) was applied as a positive control while salmon oils in the absence of LPS were considered as a negative control. Macrophage stimulation was carried out by adding 30 μL of LPS (1 mg/mL in DMEM) followed by 24 h incubation. After this period, 100 μL of the cell culture supernatants and 100 μL of the standard calibration curve (sodium nitrate, 1.6–100 mM, $y = 0.0065x + 0.1282$; $r^2 = 0.9991$) were placed into a new 96-well plate. Next, the quantification of NO was performed using a Griess Reagent System kit (Promega, Madison, WI, USA). After reaction, the absorbance of the NO produced was measured at 540 nm in the microplate reader referred above and values of the samples were compared to those of the standard. The concentrations of salmon oils needed to inhibit 50% of the NO production were determined and, therefore, the results were expressed as IC_{50} (mg/mL).

2.8.6. Antibacterial activity

Eight bacteria related to food contamination were selected to evaluate the antibacterial activity of salmon oils: *Bacillus cereus* (ATCC 11778), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 49741), *Listeria monocytogenes* (ATCC 19111), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enterica* serotype Enteritidis (ATCC 13076), *Staphylococcus aureus* (ATCC 11632), and *Yersinia enterocolitica* (ATCC 8610). All microorganisms were obtained from Frilabo (Porto, Portugal). In order to maintain the exponential growth phase, Gram-positive bacteria were incubated in fresh Blood Agar (7% sheep blood) and Gram-negative bacteria in Muller Hilton Agar, all of them at 37 °C for 24 h before the analysis. Then, bacterial suspensions were prepared at 1.5×10^6 CFU/mL.

The broth microdilution method (96-well plates) and the rapid INT colorimetric assay previously described by Pires et al. (2018) were applied to determine the antibacterial potential of the samples. Salmon oils were firstly dissolved 50% in MHB (0.5% Tween 80). Through serial dilutions, they were tested from 50 to 0.39%. Briefly, 90 μL of each oil concentration were mixed with 10 μL of bacterial suspension and the microplates were incubated under shaking at 37 °C for 18–24 h. Afterward, 40 μL of INT dye (0.2 mg/mL) was added to each well and plates were incubated again at 37 °C for 30 min. Then, bacterial growth was monitored by changing the color of viable cells from yellow to pink. The lowest concentration of oil, which showed no color change, was considered as minimum inhibitory concentration (MIC). Ampicillin (20 μg/mL) and streptomycin (1 mg/mL) were used as positive controls for all bacteria, except for *S. aureus* where methicillin (1 mg/mL) was employed. Two negative controls (MHB and fish oil sample) were also prepared for each inoculum.

The determination of the minimum bactericidal concentration (MBC) was performed by transferring 10 μL of liquid from each well that showed no color change into solid medium (Blood Agar, 7% sheep blood) and incubated at 37 °C for 24 h. MBC was defined as the lowest concentration required to kill bacteria.

2.8.7. Antifungal activity

Two foodborne fungi were used to assess the antifungal capacity of salmon oils: *Aspergillus fumigatus* (ATCC 204305) and *Aspergillus brasiliensis* (ATCC 16404). Both fungal strains were also provided by Frilabo (Porto, Portugal). The micromycetes were cultured in malt agar plates at 25 °C for 72 h. Next, the spores were recovered from the agar surface with sterile 0.85% saline containing 0.1% Tween 80 (*v/v*) and fungal spore suspensions were adjusted at 1.0×10^5 UFC/mL.

The antifungal activity was carried out in 96 well-plates by a serial dilution technique according to Heleno et al. (2013), with some modifications. Salmon oils were prepared in MEB with 0.5% Tween 80 at concentrations ranging from 50 to 0.39% (*v/v*). Briefly, 90 μL of each oil concentration were mixed with 10 μL of fungal suspension and the microplates were incubated at 25 °C for 72 h. The growth of fungi was then observed under binocular microscope and the lowest oil concentrations without visible growth were defined as MICs. The minimum fungicidal concentration (MFC) was performed using serial subculture technique from the wells where fish oils showed antifungal capacity. Two microliters were transferred to microplates containing 100 μL of MEB per well and incubated at 25 °C for 72 h followed by additional incubation (25 °C/72 h). The lowest concentration with no visible fungal growth was defined as MFC indicating 99.5% killing of the original inoculum. Ketoconazole (1 mg/mL) was used as positive control for both MIC and MFC.

2.9. Statistical analysis

Experimental data were subjected to one-way analysis of variance (ANOVA) to determine the significance 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.

Table 1
Experimental responses obtained under the extraction conditions defined by the CCRD design for the extraction oil yield from salmon side streams and statistical information of the models fitting procedure.

Runs	RSM experimental domain			Experimental response: oil yield (%)		
	Time (min)	Power (W)	Ratio (g/L)	Backbones	Heads	Viscera
1	5	243	88	34.67	38.28	49.28
2	16	243	88	43.24	37.92	48.45
3	5	807	88	42.83	32.68	56.74
4	16	807	88	43.15	40.54	66.29
5	5	243	112	39.84	38.10	57.05
6	16	243	112	36.23	37.21	61.24
7	5	807	112	38.23	30.99	58.20
8	16	807	112	35.96	34.62	65.11
9	1	525	100	35.37	30.12	52.87
10	20	525	100	38.89	32.03	65.71
11	10.5	50	100	39.78	50.63	62.89
12	10.5	1000	100	38.99	25.63	71.10
13	10.5	525	80	44.79	41.51	43.06
14	10.5	525	120	35.58	34.49	60.72
15	10.5	525	100	35.58	38.28	61.86
16	10.5	525	100	33.91	38.85	64.94
17	10.5	525	100	40.24	39.82	62.96
18	10.5	525	100	39.90	38.91	66.06
19	10.5	525	100	42.86	41.21	63.95
20	10.5	525	100	38.38	39.09	61.83
Statistical data						
Model F-value				25.40	126.70	83.41
Lack-of-fit				0.2443	0.5678	0.5857
R^2				0.9486	0.9713	0.9570
R^2_{adj}				0.9113	0.9636	0.9455
Adequate precision				19.65	34.19	31.04
Coefficient of variance (%)				3.49	1.12	2.71

3. Results and discussion

3.1. MAE process optimization

The influence of the independent variables time, microwave power, and solid/liquid ratio on the extraction of oil from salmon side streams (backbones, heads, and viscera) by MAE was studied using RSM. The results of the 20 experimental runs carried out under the CCRD design matrix for the oil content obtained from each salmon by-product are shown in Table 1. The oil yield ranged from approximately 34–45%, 26–51%, and 43–71% for backbones, heads, and viscera, respectively. In general, the combination of extraction parameters to obtain the best oil recovery was different for each sample, which revealed the influence of the matrix intrinsic nature in the MAE process, as well as the preference for conducting an optimization study (Llompart et al., 2018).

The response values in Table 1 were fitted to the second-order polynomial Eq. (1) using Design-Expert software. The significant parameters, assessed at a 95% confidence level, were used in the development of the theoretical models, as well as the non-significant ones needed for the hierarchy. Thus, a regression equation capable of explaining the effects of the independent variables of the MAE process on the oil yield response obtained from each salmon side stream was constructed. These polynomial models, expressed in coded values, are presented in Eqs. (3)–(5):

For salmon viscera:

$$Y = 63.6 + 3.0t + 3.2P + 3.4R - 1.7t^2 + 1.0P^2 - 4.8R^2 + 1.6tP - 2.5PR \quad (3)$$

For salmon backbones:

$$Y = 39.8 + 0.7t - 2.1SR - 0.8t^2 - 1.6tR \quad (4)$$

For salmon heads:

$$Y = 39.0 + 0.3t - 3.5P - 1.0R - 3.2t^2 \quad (5)$$

The coefficients of each term in the polynomial models illustrate the effect of independent variables (t, *P*, and *R*) on the oil yield response and indicate the expected change in response per unit change in factor value when the other factors are held constant. The higher the parametric value (regardless of its sign), the more significant the independent variable will be. Thus, the extraction trends are translated by the model equations complexity and the replacement of *t*, *P*, and *R* values in these models result in a theoretical value for the oil yield.

As observed in Table 1, all models presented a non-significant lack-of-fit ($p > 0.24$) and an adequate precision greater than 19, which indicates that the model equations adequately describe the effects of the independent variables on the oil yield. The variables involved in the extraction also explained the variability of the response, since R^2 and R^2_{Adj} values higher than 0.94 and 0.91, respectively, were obtained. Thus, the three models were statistically valid and suitable to navigate the design space in the optimization stage.

Information regarding the general effects of the independent variables on oil extraction from salmon side streams can be inferred from the complexity of Eqs. (3)–(5). For backbones, the variables solid/liquid ratio and time significantly affected the oil yield through liner and/or quadratic terms (Fig. 1), and also interacted significantly, while no significant effects were induced by microwave power. For heads, solid/liquid ratio was the least influential parameter on oil extraction. Increasing microwave power negatively affected oil recovery trough a linear effect, while moderate extraction times did so positively due to the notable quadratic term. Eq. (5) describing the MAE of oil from viscera was the most complex, containing linear and quadratic terms for the three factors involved in the extraction, mainly solid/liquid ratio. The interaction of microwave power with solid/liquid ratio and time also made up this model. For this matrix, higher microwave powers favored the oil recovery rate, while low values were preferable for the other two side streams, which also demanded a lower solid/liquid ratio. All these extraction trends (illustrated in Fig. 1) confirm the strong influence of the matrix type in the MAE process, as well as the importance of carrying out an optimization of this process using RSM.

The 3D response surface graphs constructed to visually interpret the effect of the time-power-solid/liquid ratio combination on the analyzed response (oil yield) are illustrated in Fig. 2. In each graph, the excluded variable was fixed at its optimal value. As already discussed, the oil extraction process from each salmon side stream showed specific extraction trends. Thus, in order to find a set of conditions that maximized the extraction, the three independent variables were set within the experimental range, while the response was set at the maximum. The model-predicted MAE conditions that maximized the oil yield to optimal values were as follows: 291.9 W for 14.6 min at 80.1 g/L for backbones; 50.0 W for 10.8 min at 80.0 g/L for heads; and 960.6 W for 14.3 min at 99.5 g/L for viscera.

3.2. Fish oil extraction yield

The oil yield results of salmon side streams after applying SE and the optimized MAE extraction conditions are shown in Table 2. The Soxhlet method resulted in extraction yields of 57 ± 1%, 56 ± 2%, and 77 ± 2% for backbones, heads, and viscera, respectively. Regarding MAE technique, oil yields were 39.4 ± 0.3% for backbones, 38 ± 1% for heads, and 71 ± 2% for viscera. For both extraction methods, the highest amount of oil was found in viscera, while there were no differences of oil content among backbones and heads. Aspevik, Thoresen, Steinsholm, Carlehög, and Kousoulaki (2021) observed similar results of lipid content in raw salmon by-products using the Bligh and Dyer method. More quantity of oil was also determined in salmon viscera than in a mixture of salmon heads and frames by Liu et al. (2020). In addition, SE with *n*-hexane as a solvent was applied to dried salmon viscera (excluding liver), obtaining an oil percentage of 65% (Rincón-Cervera, Villarreal-Rubio, Valenzuela, & Valenzuela, 2017).

In the present study, a greater amount of salmon oil was recovered

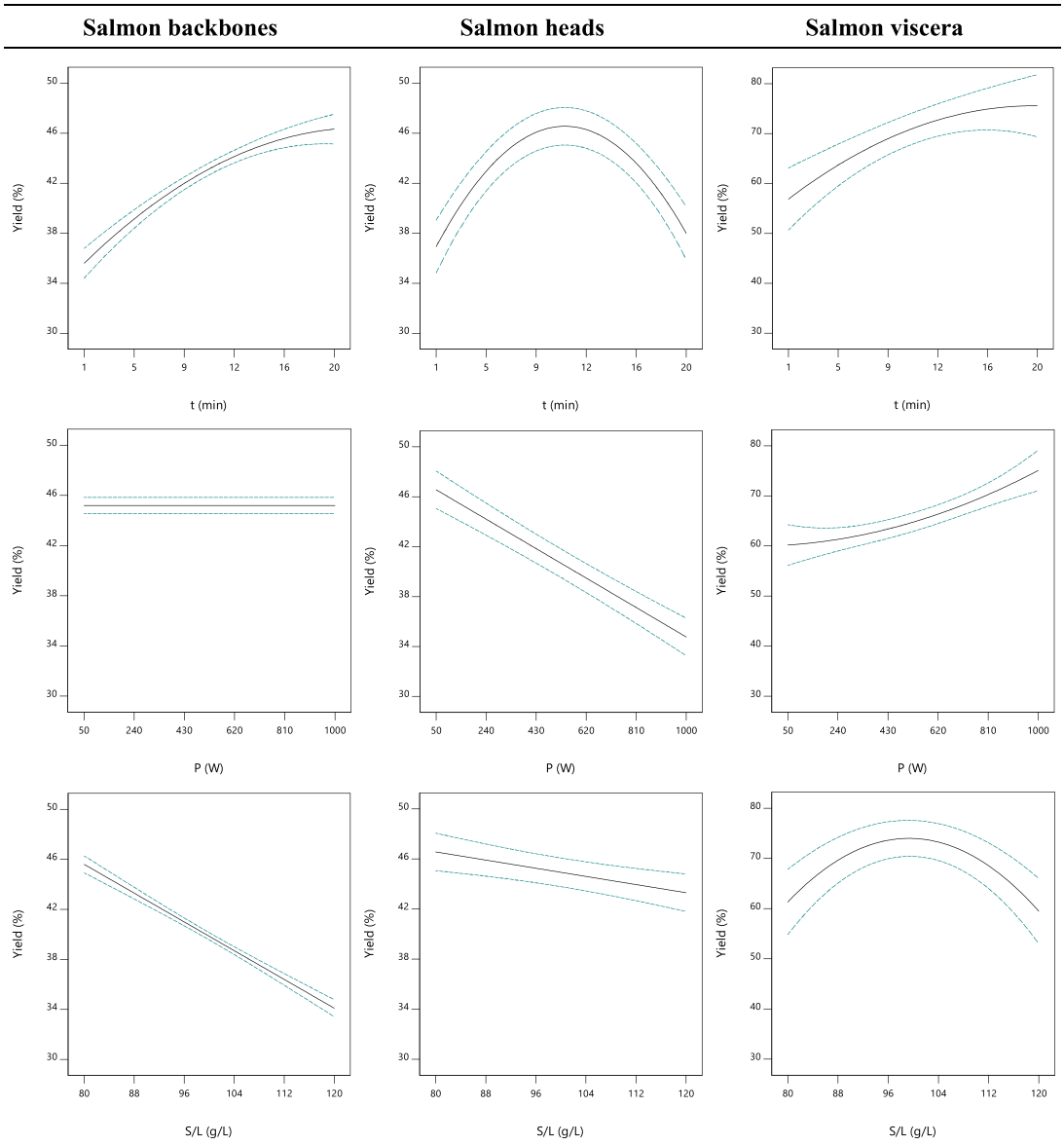

Fig. 1. 2D response graphs for the effects of the independent variables on microwave-assisted extraction of oil from salmon side streams. In each graph, the excluded variables were fixed at their optimal value.

using SE for all samples. Although MAE is considered as a novel green technology for the extraction of bioactive compounds from plants (Bagade & Patil, 2019), few studies have been carried out to extract fish oil. For instance, Ramalhosa et al. (2012) compared MAE with several traditional methods for the extraction of total lipids from edible muscle of three fish species. Their results revealed that different extraction methods provided different oil yield and MAE was considered as the technique with the best efficiency and repeatability values. Ozogul et al. (2018) also contrasted MAE, Soxhlet and Bligh and Dyer methods for fat extraction from muscle tissues of six different fishes. However, they observed comparable results between MAE and conventional techniques, concluding that the efficiency of oil extraction yield depended not only on the method applied but also on the type of fish used. In addition, Costa and Bragagnolo (2017) optimized and validated a MAE method for fish lipids using tilapia fillet. Then, optimal extraction conditions were applied to fish species with different lipid content and compared to conventional Folch method. The results did not show differences in the amount of oil obtained by MAE and Folch extraction. It is noteworthy that no information was found in the literature on the use of MAE to recover oil from fish processing by-products.

Considering SE as a reference method for the extraction of total lipid content, the optimization of MAE allowed to recover 69% of total fat content in salmon backbones and heads as well as 92% in salmon viscera, which was also carried out in <15 min for all samples. The reduction of extraction time is one of the main advantages linked to the MAE technique (Llompart et al., 2018). In this work, 360 min were required for SE while 10–14 min (depending on the sample) were used for MAE. Microwaves are supposed to cause rupture of the fish tissues, allowing the oil to be released and transferred to the solvent more quickly and efficiently than traditional methods (dos Costa & Bragagnolo, 2017). The variety of tissues that constitute fish by-products compared to fish muscle tissue could influence the oil extraction capacity in short periods of time as used here in MAE. In the case of viscera sample, it took approximately 25 times more extraction time to recover 7% more oil. Therefore, MAE could be considered as an interesting non-conventional technique to recover oil from salmon side streams, especially for salmon viscera.

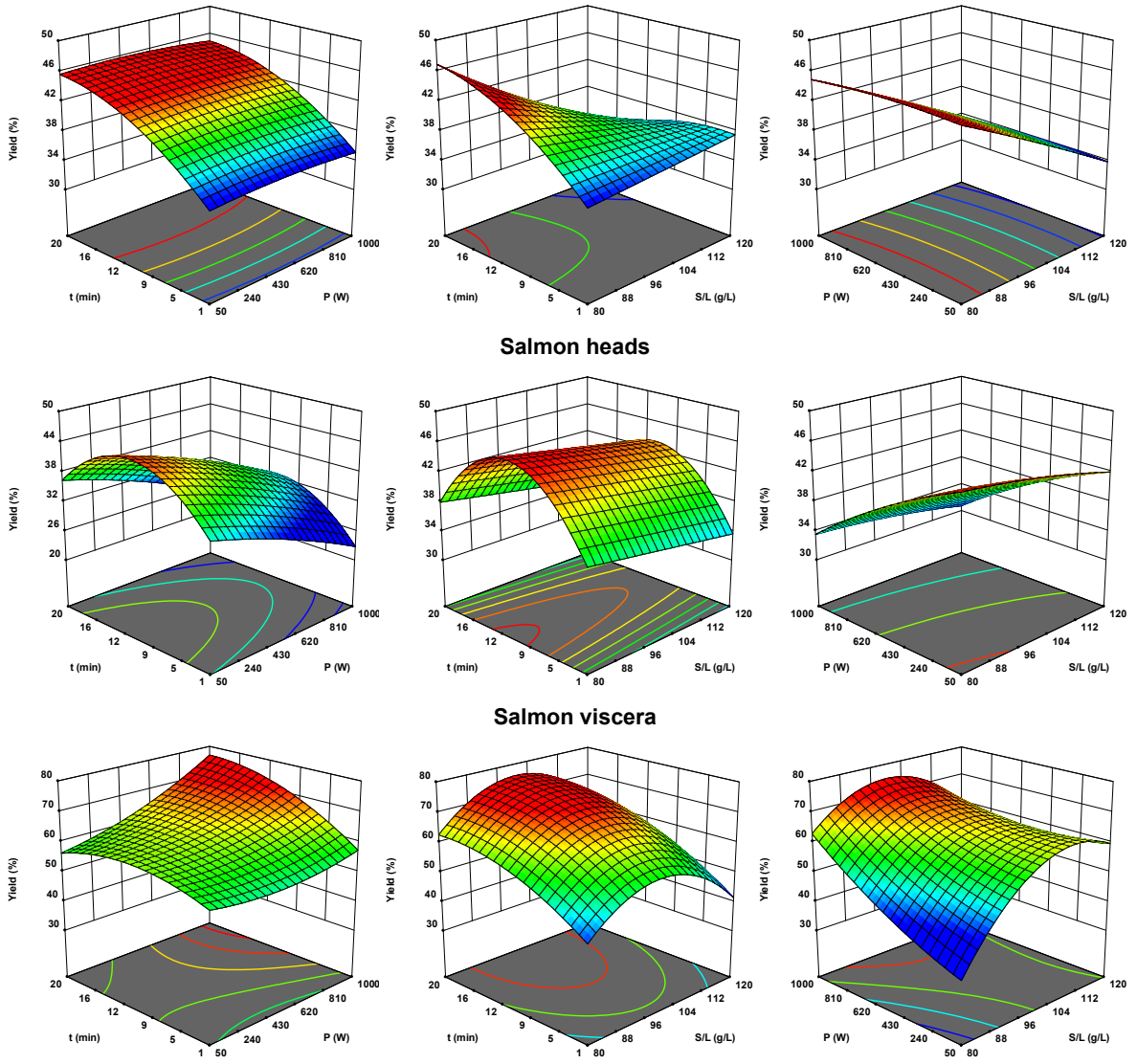

Fig. 2. Response surface graphs for the combined effects of the independent variables on oil yield (%) obtained by microwave-assisted extraction from salmon side streams. In each graph, the excluded variable was fixed at its optimal value.

3.3. Fatty acid profiles

Fatty acid composition of salmon side streams oils obtained by SE and optimal MAE are listed in Table 2. For all samples, the main fatty acids identified were oleic acid (C18:1n9c, ≈ 38%), linoleic acid (C18:2n6c, 14–18%), and palmitic acid (C16:0, ≈ 10%). Equal profiles (oleic > linoleic > palmitic) in salmon oils obtained by enzymatic hydrolysis of heads and mixture of viscera and backbones were previously reported (Liu et al., 2020). Salmon head oil extracted after heat treatment (90 °C for 1 h) and protein coagulation also showed the same lipid profile (Inguglia et al., 2020). Despite the use of different extraction techniques, the composition of the most representative fatty acids seems to remain stable in heads, viscera and backbones of Atlantic salmon. According to Rincón-Cervera et al. (2017), application of temperatures above 100 °C (not used in these studies) could affect the fatty acids that are more susceptible to thermal degradation and, therefore, modify the lipid profile. In addition, microwave energy did not alter the fatty acid composition of fish muscle (dos Costa & Bragagnolo, 2017).

Contents of saturated fatty acids (SFA, 15–17%), monounsaturated fatty acids (MUFA, 43–45%), and polyunsaturated fatty acids (PUFA, 37–41%) were similar for both extraction methods and salmon oil samples. Different percentages of the fatty acids classes of Atlantic salmon by-products have been reported (Gbogouri, Linder, Fanni, & Parmentier, 2006; Inguglia et al., 2020; Liu et al., 2020), which may due to the various extraction methods and solvents used.

The fatty acids docosahexaenoic (DHA) and eicosapentaenoic (EPA), characteristic of fatty fish, were found in the ranges of 4–6% and 7–9%, respectively. Different values of DHA and EPA in salmon side stream materials have been previously reported (Gbogouri et al., 2006; Inguglia et al., 2020; Liu et al., 2020). It should be noted that salmon viscera oil had a higher amount of linoleic and linolenic acids as well as a lower content of DHA and EPA compared to salmon head and backbone oils, which showed very few differences in their complete lipid profile. According to the European Regulation on nutritional and health claims made on foods, “high omega-3 fatty acids” content is attributed to samples with at least 80 mg of DHA + EPA/100 g of product and per 100 Kcal (Regulation (EU) No 1924/2006). Therefore, lipid fraction from salmon backbones, heads, and viscera here investigated complied with this health claim, making them interesting candidates for developing products with a healthier lipid profile through fortification.

Based on the Food and Agriculture Organization (FAO) recommendations, the values of n6/n3 ratio for human diet should be below 4

Table 2
Yield and fatty acid profile of oil extracted from salmon side streams using Soxhlet extraction (SE) and optimized microwave-assisted extraction (MAE).

	Backbones		Heads		Viscera	
	SE	MAE	SE	MAE	SE	MAE
Oil (g/100 g dw)	57 ± 1^{b}	39.4 ± 0.3 ^{a}	56 ± 2^{b}	38 ± 1 ^{a}	77 ± 2 ^{d}	71 ± 2^{c}
Fatty acid profile (%)						
C12:0	0.42 ± 0.03 ^{ab}	0.58 ± 0.02^{c}	0.36 ± 0.04 ^{a}	0.46 ± 0.01^{b}	nd	nd
C14:0	2.47 ± 0.05 ^{ab}	2.77 ± 0.05^{b}	2.5 ± 0.3 ^{ab}	2.7 ± 0.1^{b}	2.13 ± 0.09 ^{a}	2.09 ± 0.03 ^{a}
C15:0	0.18 ± 0.02 ^{a}	0.18 ± 0.01 ^{a}	0.18 ± 0.02 ^{a}	0.18 ± 0.01 ^{a}	0.17 ± 0.01 ^{a}	0.16 ± 0.01 ^{a}
C16:0	10.09 ± 0.06 ^{ab}	10.12 ± 0.06 ^{ab}	10.3 ± 0.5 ^{ab}	10.5 ± 0.1^{b}	9.9 ± 0.1 ^{ab}	9.6 ± 0.1 ^{a}
C16:1	2.51 ± 0.02 ^{a}	2.69 ± 0.01^{b}	2.59 ± 0.01 ^{ab}	2.58 ± 0.06 ^{ab}	2.05 ± 0.01 ^{a}	2.08 ± 0.06 ^{a}
C17:0	nd	nd	nd	nd	0.14 ± 0.01 ^{a}	0.14 ± 0.01 ^{a}
C17:1	nd	nd	nd	nd	0.11 ± 0.01 ^{a}	0.10 ± 0.01 ^{a}
C18:0	2.8 ± 0.1 ^{a}	2.81 ± 0.03 ^{a}	2.86 ± 0.03 ^{a}	2.9 ± 0.1 ^{a}	3.2 ± 0.1^{b}	3.00 ± 0.04 ^{ab}
C18:1n9c	38.7 ± 0.1^{c}	38.2 ± 0.2 ^{bc}	38.1 ± 0.1 ^{bc}	37.8 ± 0.3 ^{ab}	37.1 ± 0.4 ^{a}	37.7 ± 0.1 ^{ab}
C18:2n6t	nd	nd	nd	nd	0.21 ± 0.02^{b}	0.13 ± 0.04 a
C18:2n6c	14.57 ± 0.03 ^{a}	14.4 ± 0.1 ^{ab}	14.1 ± 0.2^{b}	14.1 ± 0.2 ^{ab}	18.30 ± 0.03^{c}	18.3 ± 0.1^{c}
C18:3n6	nd	nd	nd	nd	0.12 ± 0.03 ^{a}	0.14 ± 0.01^{b}
C18:3n3	7.2 ± 0.1 ^{a}	7.23 ± 0.04 ^{a}	6.99 ± 0.04 ^{a}	6.96 ± 0.01 ^{a}	8.96 ± 0.04^{b}	9.1 ± 0.1^{b}
C20:0	nd	nd	nd	nd	0.18 ± 0.01^{b}	0.14 ± 0.01 ^{a}
C20:1	2.79 ± 0.01^{c}	2.79 ± 0.04 ^{cd}	2.65 ± 0.01 ^{d}	2.71 ± 0.03 ^{d}	2.4 ± 0.1^{b}	2.20 ± 0.03 ^{a}
C20:2	1.01 ± 0.01 ^{a}	1.11 ± 0.04 ^{ab}	1.09 ± 0.03 ^{ab}	1.1 ± 0.1 ^{ab}	1.07 ± 0.01 ^{ab}	1.2 ± 0.1^{b}
C20:3n6	0.23 ± 0.01 ^{a}	0.22 ± 0.04 ^{a}	0.27 ± 0.02 ^{a}	0.25 ± 0.01 ^{a}	0.23 ± 0.01 ^{a}	0.24 ± 0.01 ^{a}
C20:4n6	0.28 ± 0.01^{b}	0.28 ± 0.01^{b}	0.31 ± 0.01^{c}	0.32 ± 0.01^{c}	0.23 ± 0.01 ^{a}	0.23 ± 0.01 ^{a}
C20:3n3	0.68 ± 0.01 ^{a}	0.64 ± 0.02 ^{a}	0.64 ± 0.01 ^{a}	0.64 ± 0.01 ^{a}	0.82 ± 0.02^{b}	0.80 ± 0.02^{b}
C22:0	0.09 ± 0.01 ^{a}	0.10 ± 0.01 ^{a}	nd	nd	0.21 ± 0.02^{b}	0.20 ± 0.01^{b}
C20:5n3	5.9 ± 0.1^{b}	5.86 ± 0.04^{b}	6.1 ± 0.3^{b}	6.16 ± 0.04^{b}	3.79 ± 0.01 ^{a}	3.72 ± 0.06 ^{a}
C22:2	0.09 ± 0.01^{b}	0.09 ± 0.01^{b}	0.07 ± 0.01 ^{a}	0.09 ± 0.01^{b}	0.09 ± 0.01^{b}	0.10 ± 0.01^{b}
C24:1	1.7 ± 0.1 ^{d}	1.57 ± 0.03 ^{cd}	1.6 ± 0.1 ^{cd}	1.5 ± 0.1 ^{bc}	1.30 ± 0.04 ^{ab}	1.29 ± 0.02 ^{a}
C22:6n3	8.2 ± 0.2^{b}	8.35 ± 0.04^{b}	9.3 ± 0.3^{c}	9.04 ± 0.07^{c}	7.3 ± 0.1 ^{a}	7.45 ± 0.02 ^{a}
Total fatty acid class						
SFA	16.1 ± 0.1 ^{a}	16.6 ± 0.2 ^{a}	16.1 ± 0.8 ^{a}	16.8 ± 0.3 ^{a}	15.9 ± 0.3 ^{a}	15.3 ± 0.1 ^{a}
MUFA	45.7 ± 0.1 ^{d}	45.2 ± 0.2 ^{cd}	44.91 ± 0.03 ^{bc}	44.6 ± 0.1^{b}	42.9 ± 0.3 ^{a}	43.3 ± 0.1 ^{a}
PUFA	37.08 ± 0.01 ^{a}	37.02 ± 0.02 ^{a}	38.8 ± 0.8^{b}	38.7 ± 0.2^{b}	41.06 ± 0.03^{c}	41.3 ± 0.1^{c}
n6/n3	0.69 ± 0.02^{b}	0.68 ± 0.01^{b}	0.64 ± 0.01 ^{a}	0.65 ± 0.01 ^{a}	0.92 ± 0.01^{c}	0.91 ± 0.01^{c}

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 eicosatrienoico; C22:0 docosanoic acid; C20:5n3 eicosapentaenoic acid (EPA); C22:2 docosadienoic acid; C24:1 nervonic acid; C22:6n3 docosahexaenoico acid (DHA); nd: not detected; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n6/n3: omega-6/omega-3 ratio. In each row, different letters mean statistical differences among samples.

Table 3
Cytotoxic, antioxidant, and anti-inflammatory activities of oil extracted from salmon side streams using Soxhlet extraction (SE) and optimized microwave-assisted extraction (MAE).

	Backbones oil		Heads oil		Viscera oil	
	SE	MAE	SE	MAE	SE	MAE
Cytotoxic activity (GI_{50} μg/mL)						
AGS	219 ± 19 ^{ab}	229 ± 14^{b}	249 ± 12 ^{bc}	286 ± 6^{c}	234 ± 15 ^{bc}	173 ± 13 ^{a}
Caco-2	297 ± 29 ^{a}	303 ± 27 ^{a}	306 ± 28 ^{a}	277 ± 25 ^{a}	363 ± 14 ^{a}	355 ± 23 ^{a}
MCF-7	205 ± 6^{b}	208 ± 19^{b}	222 ± 3^{b}	132 ± 8 ^{a}	232 ± 21^{b}	207 ± 8^{b}
NCI-H460	142 ± 12^{b}	76 ± 5 ^{a}	236 ± 18^{c}	144 ± 9^{b}	233 ± 21^{c}	217 ± 15^{c}
PLP2	201 ± 13 ^{a}	230 ± 23 ^{a}	253 ± 14 ^{a}	241 ± 2 ^{a}	249 ± 11 ^{a}	216 ± 10 ^{a}
Cellular antioxidant activity (% inhibition of oxidation at 2 mg/mL)						
RAW 264.7	nd	nd	nd	36 ± 3 ^{a}	79 ± 7^{b}	76 ± 7^{b}
Anti-inflammatory activity (IC_{50} μg/mL)						
RAW 264.7	34 ± 2 ^{a}	63 ± 3 ^{cd}	51 ± 1 ^{bc}	65 ± 5 ^{d}	42 ± 4 ^{ab}	32 ± 3 ^{a}

GI_{50} 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). IC_{50} values for Dexamethasone: 6.3 ± 0.4 μg/mL (RAW 264.7). Quercetin: 95.3 ± 4.6% oxidation inhibition at 0.3 μg/mL; wa: without activity. Different letters in each row correspond to significant differences ($p < 0.05$) among oil samples.

(Food and Agriculture Organization of the United Nations. (2010), 2010). All samples studied ranged from 0.64 to 0.92, confirming the nutritional quality of salmon by-products oils. As can be seen from Table 2, no differences in fatty acid composition were observed among Soxhlet and microwave extractions. Therefore, the conditions of power, solid/liquid ratio, and extraction time used in MAE did not influence the lipid profile of oils extracted from salmon by-products. In this sense, there were also no differences in the fatty acid profile of lipids extracted by solvent extraction and controlled proteolysis of salmon heads (Gbogouri et al., 2006).

3.4. Bioactivities of salmon oils obtained by SE and optimal MAE conditions

Data regarding cytotoxic, antioxidant, and anti-inflammatory activities of salmon oils extracted by SE and optimal MAE are presented in Table 3. All tested oils showed cytotoxic effects against all studied tumor cell lines. In general, the oils of each salmon by-product (extracted by Soxhlet and MAE) exhibited similar cytotoxicity for each cell line. Therefore, the extraction method does not seem to be decisive in the cytotoxic activity of the oils. The human adenocarcinoma cells (Caco-2) were the least susceptible to any of the tested oils. However, some studies revealed that fish-oil derived DHA reduced the viability of Caco-2 cells by increasing apoptosis and caspase-3 activity (Ahangar, Sam, Nejati, & Habibian, 2016; Jameel et al., 2019). The lung cancer cells (NCI-H460) demonstrated the highest susceptibility to the oil samples, especially for salmon backbone oil extracted by MAE ($GI_{50} = 76$ μg/mL). According to Yin, Sui, Meng, Ma, and Jiang (2017), DHA not only induced the apoptosis of non-small cell lung cancer cells *in vitro*, but also suppressed the migration and invasion of the cells. Salmon viscera oil obtained by MAE was the most effective oil ($GI_{50} = 173$ μg/mL) against

Table 4
Antibacterial activity of oil extracted from salmon side streams using Soxhlet extraction (SE) and optimized microwave-assisted extraction (MAE).

	Backbones oil				Heads oil				Viscera oil			
	SE		MAE		SE		MAE		SE		MAE	
Antibacterial activity	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria												
E. cloacae	50	wa	50	wa	50	wa	50	wa	25	wa	25	wa
E. coli	50	wa	50	wa	50	wa	50	wa	25	wa	25	wa
P. aeruginosa	50	wa	50	wa	50	wa	50	wa	50	wa	50	wa
S. enterica	50	wa	50	wa	50	wa	50	wa	50	wa	50	wa
Y. enterocolitica	50	wa	50	wa	50	wa	50	wa	3.125	wa	25	wa
Gram-positive bacteria												
B. cereus	50	wa	50	wa	50	wa	50	wa	12.5	wa	12.5	wa
L. monocytogenes	50	wa	50	wa	50	wa	50	wa	50	wa	50	wa
S. aureus	50	wa	50	wa	50	wa	50	wa	25	wa	25	wa
Antifungal activity	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
A. brasiliensis	25	wa	50	wa	25	wa	50	wa	50	wa	wa	wa
B. fumigatus	50	10	wa	wa	50	10	wa	wa	wa	wa	wa	wa

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; MFC: minimum fungicidal concentration; wa: without activity; MIC and MBC values for positive controls: streptomycin (0.007–0.01 mg/mL), methicillin (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).

the proliferation of stomach cancer cells (AGS). In this sense, different types of fish PUFAs decreased the growth of both tumor and non-tumor gastric cell lines (Dai, Shen, Pan, Shen, & Das, 2013). Among all fish oils, salmon head oil obtained by MAE exhibited the highest cytotoxicity (GI_{50} = 132 μg/mL) against the breast cancer cells (MCF-7). Fish DHA and related molecules have also showed antiproliferative effect against triple-negative, luminal, and MCF-7 breast cancer cell lines (Guo, Zhu, Wu, He, & Chen, 2017; Jameel et al., 2019). In addition, a *meta*-analysis of published research articles during 18 years concluded that fish oil consumption had a protective effect in breast cancer patients (Lachance, Radhakrishnan, Madiwale, Guerrier, & Vanamala, 2020).

Regarding the antioxidant activity, only the highest concentration (2 mg/mL) of some salmon oils were able to inhibit the oxidation reaction. The antioxidant compounds present in salmon viscera oils inhibited more than 75% of the oxidation generated in the *in vitro* macrophage cells. The slight differences in the lipid profile of salmon viscera compared to that of salmon heads and backbones (Table 2) could influence the different antioxidant activity observed. Chemical antioxidant assays have also showed the antioxidant capacity of oil from salmon belly part, trimmed muscle, backbones, and skin (Haq, Ahmed, Cho, & Chun, 2017) as well as from hake heads (Karoud et al., 2020).

As for the anti-inflammatory potential, all salmon oils demonstrated relevant NO inhibition in LPS-stimulated macrophages. The fish oil concentration needed to inhibit 50% the NO production ranged from 32 to 65 μg/mL. Ahmad, Rudd, Kotiw, Liu, and Benkendorff (2019) correlated low levels of MUFAs and high levels of EPA and DHA with inhibition of NO and TNFα in LPS-simulated macrophages, indicating that the overall lipid composition of both edible flesh and by-products from marine sources could influence the anti-inflammatory activity. Therefore, the amount of EPA and DHA found in salmon backbones, heads, and viscera here studied (Table 2) could be partly responsible for the observed anti-inflammatory effect.

Results of antibacterial and antifungal activities of salmon oils extracted by SE and optimal MAE are shown in Table 4. The backbone and head oils showed the same inhibition efficiency of bacterial growth for all strains tested without differences among extraction methods. Thus, the highest oil concentration tested (50%) displayed antibacterial activity against both Gram-positive and Gram-negative bacteria. The growth of *E. cloacae*, *E. coli*, *Y. enterocolitica*, *B. cereus*, and *S. aureus* was inhibited with lower concentrations of viscera oil (3.125–25%) than backbone and head oils (50%). There were no differences in the antibacterial activity of viscera oil depending on the extraction technique, except for *Y. enterocolitica*. The different antibacterial capacity of salmon by-products oils could be probably related to their fatty acid composition since the complete lipid profile of viscera oil differs from backbone and head oils, which were similar to each other (Table 2). Oil extracted from salmon soft tissue and salmon heads also inhibited the growth of *P. aeruginosa* and *S. aureus* (Inguglia et al., 2020). In addition, pressing and maceration methods used for the extraction of fish oil provided different activity against two bacteria strains (Simplice et al., 2018). Regarding antifungal activity and in contrast to bacterial activity, backbone and head oils appeared to have a greater effect against tested fungi compared to viscera oil. In addition, the oil extracted by Soxhlet seemed to be more effective than the oil obtained by MAE. However, to the best of our knowledge there are no reports regarding the antifungal activity of oil from fish processing by-products. Therefore, these results could be interesting for further research in this field.

4. Conclusions

The MAE technique could be considered as an interesting tool for oil extraction from salmon side streams since it allowed to recover 69% of total lipid content from backbones and heads, as well as 92% from viscera in<15 min. Salmon by-product oils have a healthy lipid profile due to their percentages of saturated and unsaturated fatty acids as well as their EPA and DHA content, making them potential candidates as ingredients in fortified food products. According to the displayed bioactivities, in particular for antimicrobial and anti-inflammatory properties, the application of salmon by-product oils could be further exploited beyond the food industry. Overall, this study shows the possibility of valorization of salmon side streams from a circular economy point of view.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.132778.

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