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Comprehensive analysis of oils and remaining solid matrices from supercritical fluid extraction of salmon side streams: Fatty acids, bioactive peptides, minerals, and heavy metals

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ABSTRACT

Atlantic salmon (Salmo salar) side streams (head, backbone, and viscera) are nutrient-rich and offer potential for valorization. This study evaluates supercritical fluid extraction (SFE) and solvent extraction (SE) methods for recovering oils, fatty acids, bioactive peptides, minerals, heavy metals, and antioxidants from these byproducts. Both the oil and the remaining solid resulting from the extraction process were analyzed. SFE was more effective in extracting fatty acids from heads and backbones. However, SE yielded a higher peptide content, with SE backbone solids containing 281 peptides, and SE viscera and heads containing 220 peptides each. In contrast, SFE-derived solids had significantly fewer peptides: 9 in heads, 129 in backbones, and 130 in viscera. The identified peptides exhibit potential functional properties, including antioxidant, anti-inflammatory, antithrombotic, immunomodulating, anticancer, and hypolipidemic effects. Mineral and heavy metal content in all extracts met safety standards. SFE-derived matrices had the highest calcium levels (49.7 mg/g in heads, 24.2 mg/g in backbones) and lower heavy metal concentrations than SE. Arsenic, cadmium, mercury, and lead levels were 1.8 mg/kg, 1.8 μg/kg, 55.0 μg/kg, and 25.7 μg/kg, respectively, for SFE, compared to SE's higher values (e.g., 3.5 mg/kg for arsenic). Both methods produced extracts with promising antioxidant activity.

1. Introduction

Global demand for Atlantic salmon (*Salmo salar*) has grown steadily (Anderson, Asche, & Garlock, 2018; Dong et al., 2024; Pandey et al., 2023), with over 90% of production concentrated in Canada, Chile, Faroe Islands, Norway, and the UK (Iversen, Asche, Hermansen, & Nystøyl, 2020). Recognized for its high level of ω-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA), particularly EPA and DHA, salmon has become a dietary staple due to its potential health benefits (Iversen et al., 2020). However, the substantial amount of discarded fish generated by the fishing industry, combined with increasing fish consumption, contributes to environmental pollution. To address this issue, researchers have advocated for utilizing fish side streams to produce or extract fish oil as a sustainable source for biodiesel production (Jaiswal, Dutta, Banerjee, V.P, & Bhushan, 2022). Moreover, recent research emphasizes that fish side streams are a potential source of health and biologically active compounds, with the capacity to yield new and valuable food ingredients (Nawaz et al., 2020).

Salmon side streams are rich in bioactive compounds and have the potential to produce new food ingredients. Several methods have been used to recover oils from salmon, including traditional solvent extraction (SE) with n-hexane (Kumar et al., 2017), although concerns over toxicity and environmental impact have driven interest in alternatives (Chemat et al., 2020). Supercritical fluid extraction (SFE) is considered eco-friendly, chemical-free, and an effective solution in recovering marine oils for functional foods, emphasizing the significance of

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omega-3 and polyunsaturated fatty acids (Chemat et al., 2020; Sultana, Adachi, & Yoshii, 2023).

Previous studies have also shown SFE's commercial potential (Mazzutti, Pedrosa, & Salvador Ferreira, 2021), but little attention has been given to the remaining solid matrices obtained after SFE extraction. These solid matrices, as well as the oil extracts, are rich in essential minerals (Smith, Etienne, & Montanari, 2024), like calcium, potassium, phosphorus, and iron, which are important for human health. Additionally, proteins and bioactive peptides recovered from these side streams offer benefits such as antioxidant, antihypertensive, and antimicrobial properties (Ucak et al., 2021).

Bearing in mind the above mentioned considerations, the primary objective of the present study is to investigate the impact of a conventional solvent extraction (SE) process and supercritical fluid extraction (SFE) on the recovery of oils and solids rich in nutrients and bioactive compounds from salmon side streams. Apart from the oil extraction yield, the fatty acid profile, mineral composition, proteins, bioactive peptides, and heavy metal profiles in both oil extracts and solid matrices will be evaluated using advanced tools.

Therefore, this study hypothesizes that using advanced extraction technologies can enhance the recovery of various nutrients and bioactive compounds by incorporating specific factors, such as applied pressure and environmentally friendly solvents like CO_2. Additionally, it is proposed that utilizing the solid material remaining after extraction, rather than relying solely on the recovered oil, could enable a zero-waste approach. This comprehensive use of the entire biomass may provide a foundation for future applications in food production.

2. Materials and methods

2.1. Samples

The freeze-dried salmon side streams (heads, backbone, and viscera) were provided by the Department of Nutrition and Feed Technolo gy of the Nofima Food, fisheries and Aquaculture Research Institute (Bergen, Norway). The freeze-dried salmon side streams were kept at −25 °C until further analysis. The treatment, extraction, and identification of salmon side streams using the SFE and SE methods are presented in Fig. 1.

2.2. Chemicals and reagents

The 2,2′-azinobis-3-ethylbenzthiazoline-6-sulphonate (ABTS) was purchased from Sigma-Aldrich (Steinheim, Baden-Württemberg, Germany). The deionized water was obtained from the Milli-Q SP reagent water system (Millipore Corporation, Bedford, MA, USA). The sulfuric acid (98%), *n*-hexane (95%), and methanol were purchased from Fisher Scientific (Leicestershire, UK). Titrisol concentrated standards of minerals (Mg, Ca, P, Fe, Zn, Se), heavy metals solution standards and nitric acid (HNO_3) were purchased from Merck (Darmstadt, Germany). The fatty acid methyl ester (FAME) reference standard mixture 37 was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Solvent (SE) and supercritical fluid (SFE) extraction

The SE was performed according to the previous method with few adjustments (Haq, Ahmed, Cho, & Chun, 2017; Rincón Cervera, Venegas, Ramos Bueno, Suárez; Medina, & Guil Guerrero, 2015; Sahu, Kundu, & Sethi, 2021). Firstly, 3 g of freeze-dried salmon side streams and 20 mL of *n*-hexeze were mixed in a beaker and stirred at 300 rpm for 30 min at 25 °C. Then, the sample was centrifuged at 4000 rpm for 15 min at 4 °C (Centrifuge 5810R, Eppendorf, Germany) and the supernatant was collected. 15 mL *n*-hexane was added to the solid extracts and stirred at 300 rpm at 25 °C for the second time extraction. The supernatant and solid extracts were collected again after centrifuged. Both supernatants were combined, and then nitrogen blowing (TurboVap Evaporator, Zymark Corporation, Canada) was utilized to remove the *n*-hexane, and the extracted oil was collected and stored at −20 °C until

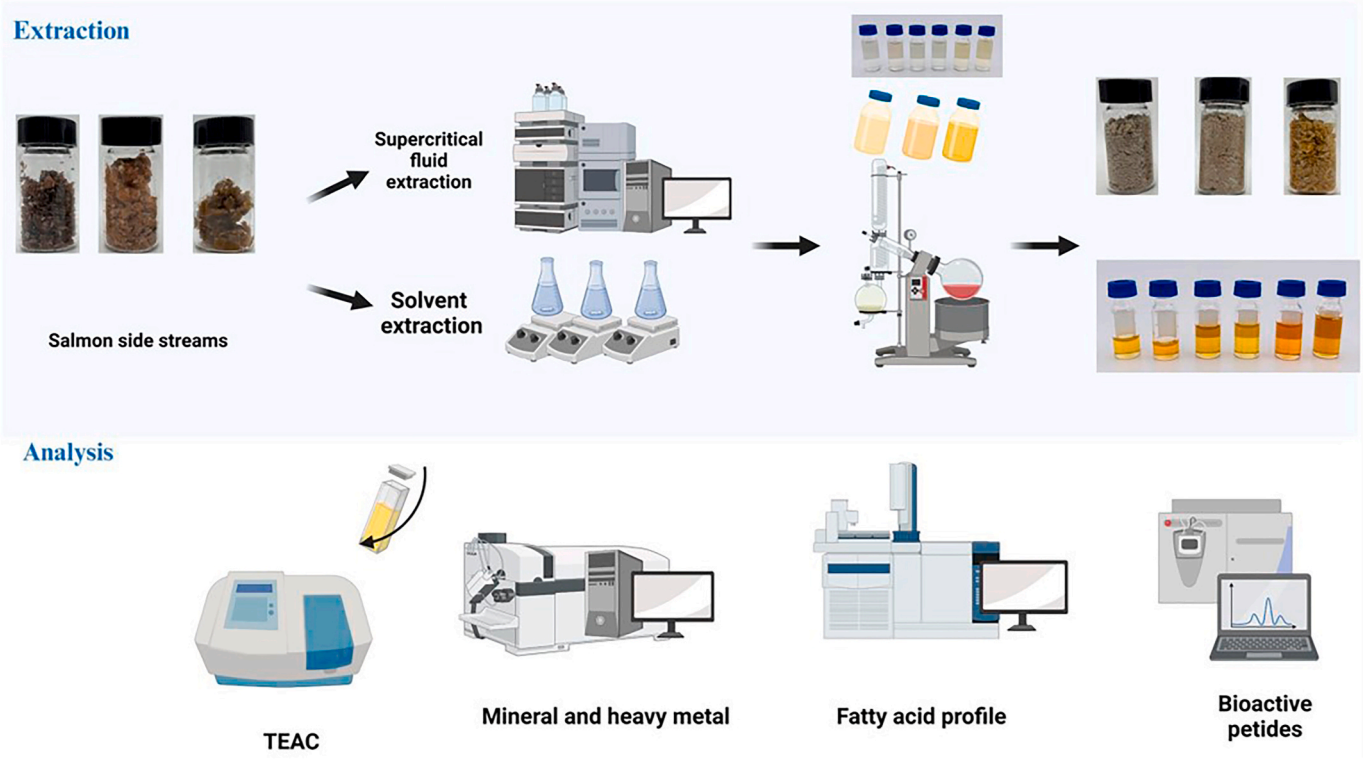

Fig. 1. The extraction and identification process of salmon side streams using the supercritical fluid extraction (SFE) and solvent extraction (SE) methods (Created with BioRender.com).

further analysis. All extractions were carried out in triplicates.

The SFE was applied to recover the oil compounds from the salmon side streams. SFE was operated using the system (JASCO, Tokyo, Japan) located in the lab of the ALISOST team at the Faculty of Pharmacy and Food Sciences (University of Valencia, Valencia, Spain) based on the previous method with some modifications (Salgado-Ramos et al., 2023). Three grams of freeze-dried salmon side streams (head, backbone, or viscera) were packed into the 25 mL vessel (803559-25 mL) for extraction. The process conditions were as follows: the working temperature was 40 °C, the extraction time was 75 min, the pressure was 25 MPa, followed by the dynamic extraction at the 10 mL/min flow (0.8 mL/min of ethanol absolute and 9.2 mL/min of CO_2). All extractions were carried out in triplicate.

2.4. Oil yield determination

The salmon side stream extraction was performed using the SE and SFE methods. The liquid extracts were collected, and then nitrogen blowing (TurboVap Evaporator, Zymark Corporation, Canada) was performed to obtain the oil extracts. The extracted salmon oil was considered as the total oil compound, and the extraction yield of oil was calculated referenced to the previous method (de la Fuente et al., 2022) according to equation (1) as follows:

$$\text{Extraction yield of oil}\ (\%) = \frac{\text{Weight of obtained oil}}{\text{Weight of sample}\ (\text{dry basis})} \times 100 \qquad (1)$$

2.5. Fatty acid profiles analysis

The fatty acid profiles were carried out using a previous method described by Martí-Quijal et al.(Martí Quijal, Pallarés, Dawidowicz, Ruiz, & Barba, 2023(Martí Quijal, Pallarés, Dawidowicz, Ruiz, & Barba, 2023). To sum up, 1 mL of the extract was mixed with a mixture of 1 mL of 2N KOH in methanol and 1 mL of hexane. After stirring the mixture for 2 min, the hexane phase was extracted, and 2 μL of this phase was injected into a PerkinElmer Gas Chromatograph Clarus® 590. The fatty acids were identified using a standard FAME Mix (Supelco 37 Component FAME Mix, Sigma-Aldrich, Laramie, WY, USA). The determination was performed by Gas chromatography coupled to a flame ionization detector (GC-FID, GC-Agilent 7890B, Agilent Technologies, Santa Clara, CA, USA). The GC column had a dimension of 30 m length x 0.25 mm internal diameter x 0.25 μm thick cover (Agilent Technologies, Santa Clara, CA, USA). The oven temperature was set at 180 °C for 5 min; then the temperature was increased up to 210 °C at the rate of 4 °C/min; subsequently, the temperature rose to 250 °C at the rate of 10 °C/min and remained at this high temperature for 20 min. Nitrogen was used as the carrier gas at the flow rate of 20 PSI.

2.6. Determination of minerals and heavy metals by inductively coupled plasma mass spectrometry (ICP-MS)

All the solid matrices were freeze-dried (Model 10N, Gedilab S.L., Spain) at −61 °C for 72 h and then crushed into the sample powder using the Pulverisette 11 (Fritsch, Germany) for 3 min (200–240 V in voltage, 50/60 Hz in frequency, 1250 W in power). The ICP-MS (ICP-MS7900, Agilent Technologies, Santa Clara, CA, USA) identification and determination of minerals and heavy metals was performed in the solid matrices and oil extracts. The detailed method and conditions were referenced to the prior method described in detail by Wang et al. (M. Wang et al., 2023).

2.7. Protein content

The protein content was analyzed by the Dumas method (Liu et al., 2024). The total protein content profiles of the solid matrices were obtained by multiplying nitrogen content by the conversion factor of 6.25.

2.8. Bioactive peptides (BPs)

Bioactive peptides have been identified in the proteomics facility of SCSIE University of Valencia using an LC/MS Q-TOF system (6600plus Triple TOF, ABSCIEX, Framingham, MA, USA). The detailed information has been described by previous work (Martí Quijal, Castagnini, Ruiz, & Barba, 2023). Samples were centrifuged at 15,000 rpm for 6 min, and supernatants were collected. Peptides were precipitated by adding acetonitrile (ACN) in a 1:1 ratio and incubated overnight. After centrifugation at 5000 rpm for 5 min, supernatants were dried in a speed vacuum, and pellets were reconstituted in 27 μL of 2% ACN and 0.1% trifluoroacetic acid (TFA), followed by 5 min of sonication. Peptide concentrations were measured at 280 nm using a nanodrop. Each sample was diluted in TFA and ACN, and 5 μL was loaded onto a ChromXP C18 trap column for desalting at a 5 μL/min flow rate. Peptides were then transferred to a pre-equilibrated 3 μm C18-CL analytical column and eluted over 20 min with a 5%–75% B gradient at 300 nL/min. Analysis was performed on a nanoESI qQTOF mass spectrometer, ionized at 3.0 kV and 175 °C. MS1 scans (350–1400 m/z) were captured for 250 ms, and MS2 scans (100–1500 m/z) for 25 ms, with a 15-s dynamic exclusion. System sensitivity was evaluated using 0.5 μg of K562 trypsin digestion. Peak lists were generated using ProteinPilot standard parameters. Besides, the potential bioactivity for various peptides (including the various peptides' activity, peptides' sequence, sequence of activity, and location) was identified using the BIOPEP-UWM DATABASE (Minkiewicz, Iwaniak, & Darewicz, 2019).

2.9. Antioxidant capacity using Trolox equivalent antioxidant capacity (TEAC) assay

The scavenging activity was studied for the SFE/SE liquid extracts according to the previous method with some modifications (Shen et al., 2023; M. Wang et al., 2021; Zhang, Yu, & Zhao, 2023). The absorbance was then measured at 734 nm and the TEAC was performed with UV spectroscopy (PerkinElmer). The initial solution was prepared with the 25 mL ABTS (7 mM) and 440 μL $K_2S_2O_8$ (140 mM) and stored in the darkness for 16 h. This solution was diluted to create a working solution. For the assay, 2.5 mL of the working solution was combined with 100 μL of appropriately diluted samples, and absorbance was measured after a 3-min reaction period. The antioxidant capacity was calculated using a standard curve.

2.10. Statistical analysis

All tests were conducted with at least three replicates, and mean values were presented. The statistical differences in the data were determined using a one-way analysis of variance (ANOVA) and Duncan's multiple range test with significant differences ($p < 0.05$). Data distribution was evaluated using the Shapiro-Wilk test. GraphPad Prism (GraphPad Software Company, La Jolla, CA, USA) was used for graph design, and SPSS 20.0 analysis software (IBM Cop., Armonk, NY, USA) was used to perform statistical analyses.

3. Results and discussion

3.1. Oil yield

The oil yield serves as a crucial indicator for evaluating the efficacy of several extraction methods. In our study, *n*-hexane was selected as the reference and conventional solvent for extracting oil from the salmon side stream for comparison purposes with the innovative technology used (SFE). This choice is based on its ability to meet desirable conditions, such as a low boiling point, which facilitates its removal post-extraction, and a significantly different density compared to water (Mercer & Armenta, 2011). The results of the extraction yield of oil recovered from the salmon side streams assisted by *n*-hexane and SFE

are presented in Fig. 2.

As can be observed in the figure, oil yields of 51.5% for the salmon head, 48.3% for the backbone, and 70.1% for viscera were obtained after SE (*n*-hexane performed as the solvent), while SFE yielded 51.7%, 51.9%, and 65.6% oil recovery, for the salmon head, backbone, and viscera, respectively. Although non-significant differences in the oil recovery were observed for the salmon head, regardless of the treatment applied, a significant increase in the oil recovery of salmon backbone was observed when SFE was used as an extraction technique while higher oil yield values were obtained after the application of SE to viscera compared to SFE. Although SFE is regarded as a greener and more innovative technique, the results demonstrated higher extraction outcomes, particularly for the salmon backbones samples. Similar findings have been previously reported (Ferdosh et al., 2013). For instance, fish oil from common carp (*Cyrpinus carpio* L.) tissues obtained by SFE (40 MPa, 60 °C) were tested as a source of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (Kuvendziev, Lisichkov, Zeković, Marinkovski, & Musliu, 2018). Moreover, in another study evaluating the influence of process parameters on the extraction yield and oil quality derived from Hake (*Merluccius capensis–Merluccius paradoxus*) side streams, more than 96% of the total oil (6% EPA and 14% DHA) contained in the raw material was extracted after 3 h under the optimal SFE conditions (25 MPa, 10 kg CO_2/h, and 40 °C) (Rubio-Rodríguez et al., 2008).

3.2. Fatty acid profile

Twenty-six fatty acids were found in the oil samples obtained from salmon side streams (head, backbone and viscera) (Table 1), being oleic acid (C18:1 cis(n9), 33.13–46.18 mg/g dry weight (DW)), arachidic acid (C20:0, 12.30–19.78 mg/g DW), and palmitic acid (C16:0, 6.23–8.18 mg/g DW), the predominant ones. These findings fully agree with the profiles and contents previously obtained by de la Fuente et al. (de la Fuente et al., 2022) when they recovered oils from salmon side streams. Fish oil is considered highly significant for functional foods or direct incorporation into food, serving as a crucial source of n-3 fatty acids (omega-3) and polyunsaturated fatty acids (PUFAs) (Sultana et al., 2023). For instance, some previous studies have highlighted the vital role of polar lipids in brain bioavailability, particularly concerning placental transfer and the blood-brain barrier, where the deposition of complex lipids significantly contributes to rapid brain growth (Zheng, Fleith, Giuffrida, O'Neill, & Schneider, 2019).

Regarding salmon heads and backbones, the SFE process demonstrated a superior efficiency in the recovery of fatty acids compared to SE. For instance, the fatty acid concentrations of salmon heads and backbones increased ($p < 0.05$) after the application of SFE compared to SE. The apparent differences were especially observed in salmon heads for the three main fatty acids found, obtaining the values of 42.27,

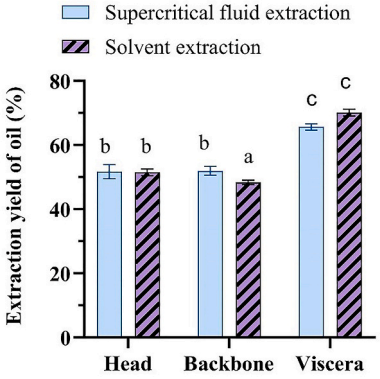

Fig. 2. Extraction yield of oil recovered from salmon side streams using the supercritical fluid extraction (SFE) and solvent extraction (SE) methods.

Table 1
Fatty acid profiles of salmon side streams oil extracted with supercritical fluid extraction (SC-CO_2) and solvent extraction (SE) methods.

Fatty acid (mg/g DW)	SE head	SC-CO_2 head	SE backbone	SC-CO_2 backbone	SE viscera	SC-CO_2 viscera
C12:0	0.35 ± 0.02 ^{b}	0.42 ± 0.01 ^{c}	0.38 ± 0.03 ^{b}	0.60 ± 0.04 ^{d}	nd	0.04 ± 0.00 ^{a}
C13:0	nd	nd	nd	nd	nd	nd
C14:0	2.23 ± 0.02 ^{a}	2.91 ± 0.08 ^{b}	2.2 ± 0.19 ^{a}	2.9 ± 0.24 ^{b}	2.28 ± 0.37 ^{a}	2.3 ± 0.21 ^{a}
C14:1	nd	nd	nd	nd	nd	nd
C15:0	0.13 ± 0.00 ^{a}	0.17 ± 0.00 ^{c}	0.13 ± 0.01 ^{a}	0.17 ± 0.02 ^{c}	0.16 ± 0.02 ^{b}	0.15 ± 0.01 ^{b}
C15:1	nd	nd	nd	nd	nd	nd
C16:0	6.27 ± 0.08 ^{a}	8.18 ± 0.08 ^{b}	6.23 ± 0.53 ^{a}	7.79 ± 0.61 ^{b}	8.10 ± 1.25 ^{b}	7.99 ± 0.42 ^{b}
C16:1	nd	nd	0.16 ± 0.00 ^{a}	0.21 ± 0.01 ^{a}	3.10 ± 0.47 ^{b}	3.05 ± 0.17 ^{b}
C17:0	2.81 ± 0.03 ^{a}	3.61 ± 0.05 ^{b}	2.70 ± 0.29 ^{a}	3.56 ± 0.29 ^{b}	nd	nd
C17:1	0.17 ± 0.01 ^{a}	0.26 ± 0.00 ^{c}	0.19 ± 0.03 ^{ab}	0.27 ± 0.04 ^{c}	0.22 ± 0.02 ^{b}	0.21 ± 0.01 ^{b}
C18:0	2.58 ± 0.04 ^{a}	3.3 ± 0.08 ^{b}	2.63 ± 0.32 ^{a}	3.24 ± 0.32 ^{b}	3.86 ± 0.53 ^{c}	3.7 ± 0.18 ^{c}
C18:1 trans (n9)	nd	nd	nd	nd	nd	0.1 ± 0
C18:1 cis (n9)	33.93 ± 0.31 ^{a}	42.27 ± 0.64 ^{b}	34.52 ± 3.10 ^{a}	42.83 ± 3.40 ^{b}	46.18 ± 6.80 ^{b}	45.09 ± 2.20 ^{b}
C18:2 trans (n6)	nd	nd	nd	nd	nd	nd
C18:2n6c cis (n6)	nd	0.15 ± 0.02 ^{ab}	0.11 ± 0.05 ^{a}	0.19 ± 0.03 ^{b}	nd	nd
C20:0	12.30 ± 0.08 ^{a}	14.57 ± 0.24 ^{bc}	12.58 ± 1.14 ^{ab}	15.57 ± 0.8 ^{c}	19.78 ± 3.46 ^{d}	19.1 ± 1.73 ^{d}
C18:3n6	nd	0.16 ± 0.00 ^{cd}	0.12 ± 0.02 ^{a}	0.18 ± 0.02 ^{d}	0.16 ± 0.01 ^{c}	0.14 ± 0.01 ^{b}
C20:1(n9)	2.60 ± 0.19 ^{a}	3.46 ± 0.05 ^{d}	2.88 ± 0.24 ^{ab}	3.34 ± 0.26 ^{cd}	3.34 ± 0.46 ^{cd}	3.11 ± 0.04 ^{bc}
C18:2n6t cis (n6)	3.33 ± 0.16 ^{a}	3.99 ± 0.08 ^{b}	3.29 ± 0.35 ^{a}	4.34 ± 0.34 ^{b}	5.08 ± 0.76 ^{c}	5.05 ± 0.35 ^{c}
C21:0	nd	nd	nd	0.07 ± 0.00 ^{a}	nd	nd
C20:2	1.81 ± 0.00 ^{a}	2.01 ± 0.08 ^{ab}	1.89 ± 0.19 ^{a}	2.33 ± 0.04 ^{bc}	2.37 ± 0.55 ^{c}	2.24 ± 0.15 ^{bc}
C22:0	nd	nd	nd	nd	nd	nd
C20:3n6	nd	0.29 ± 0.04 ^{a}	nd	nd	nd	nd
C22:1n9	1.93 ± 0.08 ^{a}	2.37 ± 0.21 ^{b}	2.05 ± 0.18 ^{a}	2.36 ± 0.02 ^{b}	2.00 ± 0.34 ^{a}	1.87 ± 0.08 ^{a}
C20:3n3	0.30 ± 0.04 ^{a}	0.33 ± 0.00 ^{ab}	0.29 ± 0.03 ^{a}	0.36 ± 0.02 ^{b}	0.45 ± 0.09 ^{c}	0.43 ± 0.02 ^{c}
C23:0	nd	nd	nd	nd	nd	nd
C20:4n6 (ARA)	0.31 ± 0.02 ^{cd}	0.35 ± 0.01 ^{ed}	0.28 ± 0.03 ^{bc}	0.37 ± 0.01 ^{e}	0.24 ± 0.07 ^{ab}	0.23 ± 0.00 ^{a}

(*continued on next page*)

Table 1 (*continued*)

Fatty acid (mg/g DW)	SE head	SC-CO_2 head	SE backbone	SC-CO_2 backbone	SE viscera	SC-CO_2 viscera
C22:2	0.88 ± 0.05 ^{a}	1.03 ± 0.00 ^{bc}	0.87 ± 0.09 ^{a}	1.12 ± 0.10 ^{c}	0.94 ± 0.16 ^{ab}	0.87 ± 0.05 ^{a}
C24:0	nd	0.10 ± 0.01 ^{a}	0.09 ± 0.00 ^{a}	0.11 ± 0.01 ^{ab}	0.17 ± 0.06 ^{c}	0.15 ± 0.00 ^{bc}
C20:5n3 (EPA)	3.09 ± 0.01^{b}	4.02 ± 0.08 ^{c}	2.96 ± 0.22 ^{b}	3.99 ± 0.32 ^{c}	2.07 ± 0.34 ^{a}	1.98 ± 0.12 ^{a}
C24:1	0.35 ± 0.03 ^{a}	0.44 ± 0.01 ^{abc}	0.35 ± 0.00 ^{a}	0.45 ± 0.03 ^{bc}	0.52 ± 0.16 ^{c}	0.42 ± 0.02 ^{ab}
C22:6(n3) (DHA)	4.27 ± 0.08 ^{a}	5.53 ± 0.04 ^{b}	4.01 ± 0.30 ^{a}	5.53 ± 0.50 ^{b}	4.14 ± 0.85 ^{a}	3.74 ± 0.29 ^{a}

Data are expressed as means ± SD. Values followed by different superscripts in the same column present significantly different ($P < 0.05$).
C12:0, lauric acid; C 13:0, tridecanoic acid; C14:0, myristic acid; C 14:1, myristoleic acid; C15:0, pentadecanoic acid; C 15:1, *cis*-10-pentadecenoic acid; C16:0, palmitic acid, C16:1, palmitoleic acid; C17:0, heptadecanoic acid; C17:1, *cis*-10-heptadecenoic acid; C18:0, stearic acid; C18:1 cis (n9), oleic acid; C18:1 trans (n9), elaidic acid; C18:2 cis (n6), linoleic acid; C18:2 trans (n6), linolelaidic acid; C18:3n6, γ-linoleic acid; C18:3n3, α-linolenic acid; C20:0, arachidic acid; C20:1(n9), *cis*-11-eicosenoic acid; C20:2, *cis*-11,14-eicosadienoic acid; C20:3n6, *cis*-8,11,14-eicosadienoic acid; C20:3n3, *cis*-11-14,17-eicosatrienoico acid; C20:4n6 (ARA), arachidonic acid; C20:5n3 (EPA), *cis*-5,8,11,14,17-eicosapentaenoic acid; C21:0, henicosanoic acid; C22:0, behenic acid; C22:1n9, erucic acid; C22:2, *cis*-13,16-docosadienoic acid; C22:6n3 (DHA), *cis*-4,7,10,13,16,19-docosahexaenoico acid; C23:0, tricosanoic acid; C24:0, lignoceric acid; C24:1, nervonic acid; nd: not detected. In each row, different letters mean statistical differences among samples.

14.57, and 8.18 mg/g DW for C18:1cis(n9), C20:0, and C16:0, respectively, while the concentrations for the same matrix after SE were 33.93, 12.30, and 6.27 mg/g DW for C18:1cis(n9), C20:0, and C16:0, respectively. Similarly, the backbone oil concentrations of C18:1cis(n9), C20:0, and C16:0 after the application of SFE were 42.83, 51.57, and 7.79 mg/g DW, while for SE method the values obtained were 34.52, 12.58, 6.23 mg/g DW, respectively.

The highest quantity of C18:1cis(n9) (46.18 mg/g DW) was found for the oil recovered from viscera after the application of SE, followed by the SFE (45.09 mg/g DW). In terms of the salmon viscera, the fatty acids did not display a noticeable difference ($p > 0.05$) between the SE and SFE methods (Table 1). The viscera results presented in Table 1 might imply that the SFE did not promote an apparent enhancement in the fatty acid profile extraction compared to the SE. Typically, the fish viscera contain an abundant quantity of fatty acids, which was also corroborated in this study, showing a significant amount of C18:1cis(n9) (46.18 mg/g DW).

Omega-3 fatty acids play a crucial role in benefiting both the eyes and brain in humans, exhibiting preventive functions against certain diseases such as cardiac issues and cerebral complaints (Pradeepkiran, 2019). The well-known fatty acids, DHA and EPA, were identified in the salmon side streams, with profiles ranging from 3.74 to 5.73 mg/g DW and 1.98–3.99 mg/g DW, respectively. These results are in full agreement with previous studies (de la Fuente et al., 2022).

Regarding DHA and EPA, both SE viscera and SFE viscera exhibited lower values compared to the SE head and SE backbones. This emphasizes the considerable potential of the head and backbone to produce essential fatty acids like DHA and EPA. The concentrations in viscera were also lower than those in salmon head and backbone, aligning with previous research findings (de la Fuente et al., 2022). In conclusion, the rich unsaturated fatty acid composition of oils extracted from salmon side streams suggests their potential health benefits for consumers.

3.3. ICP-MS analysis of heavy metals

The contamination of heavy metals presents a significant threat to aquatic environments and their inhabitants, particularly when concentrations exceed safe limits. Heavy metals, due to their non-biodegradable nature and prolonged persistence, present toxicity risks to fish and other aquatic organisms (Taslima et al., 2022). Fish, being a valuable resource of high biological significance, serve as robust models for assessing the risks and benefits associated with contaminants on human health, among various available species (Marengo et al., 2018). In this study, we investigated the concentrations of arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) in both solid matrices and oil extracts obtained from salmon side streams, being the results presented in Fig. 3. The various matrices derived from salmon side streams exhibited different profiles and concentrations of As, Cd, Hg, and Pb, reflecting significant differences among them.

From the results obtained, it was found that the concentrations of As, Cd, Hg, and Pb in the remaining solid matrices after SFE were 1.8 mg/kg, 1.8 μg/kg, 55.0 μg/kg, and 25.7 μg/kg, while the values obtained for the remaining solid matrices after using SE were 3.5 mg/kg, 4.0 μg/kg, 70.0 μg/kg, and 33.1 μg/kg for As, Cd, Hg, and Pb, respectively. The values obtained showed that lower heavy metal concentrations can remain in the solid matrices after using SFE compared to the SE. The Cd, Hg, and Pb concentration in solid matrices were lower than those found in the oil samples, indicating that greater amounts of Cd, Hg, and Pb was extracted from the salmon side streams into the oil extracts, remaining lower concentrations in the solid matrices. The concentrations in the oil extracts for As, Cd, Hg, and Pb in the solid matrices after applying SFE were 1.2 mg/kg, 1.3 μg/kg, 4.4 μg/kg, and 11.1 μg/kg, while the values obtained after using SE were 0.6 mg/kg, 0.8 μg/kg, 3.5 μg/kg, and 13.9 μg/kg. Contrary to the SE, the oil extracts obtained after SFE had higher values of As, Cd, and Hg.

Regarding backbones, the values obtained for As, Cd, Hg, and Pb in the remaining solid matrices obtained by SFE were 1.3 mg/kg, 1.1 μg/kg, 44.0 μg/kg, and 10.9 μg/kg, while the concentrations after using the SE were 3.0 mg/kg, 7.7 μg/kg, 61.1 μg/kg, and 799.0 μg/kg. Lower values of As, Cd, Hg, and Pb were found in the solid matrices after SFE, thus indicating that more heavy metals were extracted into the oil extracts from the salmon backbone tissue. The concentrations of As, Cd, Hg, and Pb in the SFE oil extracts were 0.6 mg/kg, 2.4 μg/kg, 4.3 μg/kg, and 10.9 μg/kg, while the concentrations in the oils obtained after using the conventional extraction were 0.5 mg/kg, ND, ND, and 8.7 μg/kg.

Regarding viscera, the contents of As, Cd, Hg, and Pb in the solid matrices obtained after SFE were 1.8 mg/kg, 49.5 μg/kg, 74.0 μg/kg, and 163.0 μg/kg, while the values using the conventional extraction were 2.8 mg/kg, 65.2 μg/kg, 112.0 μg/kg, and 110.0 μg/kg, respectively. Furthermore, lower concentrations were found for these heavy metals in the oil extracts compared to the remaining solid matrices. As for the oil extracts, the concentrations for As, Cd, Hg, and Pb after SFE were 0.6 mg/kg, 0.8 μg/kg, 4.3 μg/kg, and 20.9 μg/kg, while the values using the SE were 0.5 mg/kg, 1.2 mg/kg, 1.5 mg/kg, and 60.0 μg/kg. A significantly decreased amount of Cd and Pb after SFE process was found compared to conventional extraction (SE).

Previous researchers concluded that, regarding the maximum permitted levels for the As, Cd, chromium (Cr), copper (Cu), Fe, nickel (Ni), Pb, and Zn, the regulatory limit is equal to the 13.5 mg/kg, 0.05 mg/kg, 4.0 mg/kg, 20.0 mg/kg, 10.2 mg/kg, 80.0 mg/kg, 0.3 mg/kg and 50 mg/kg of wet fish tissue, respectively (Kandyliari et al., 2021; Renieri et al., 2019). Therefore, the results obtained in this study underscore the potential safe utilization of salmon side streams, both the oil extracts and the remaining solid matrices obtained by SFE and SE, in the food industry.

3.4. ICP-MS analysis of minerals

Magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), Fe,

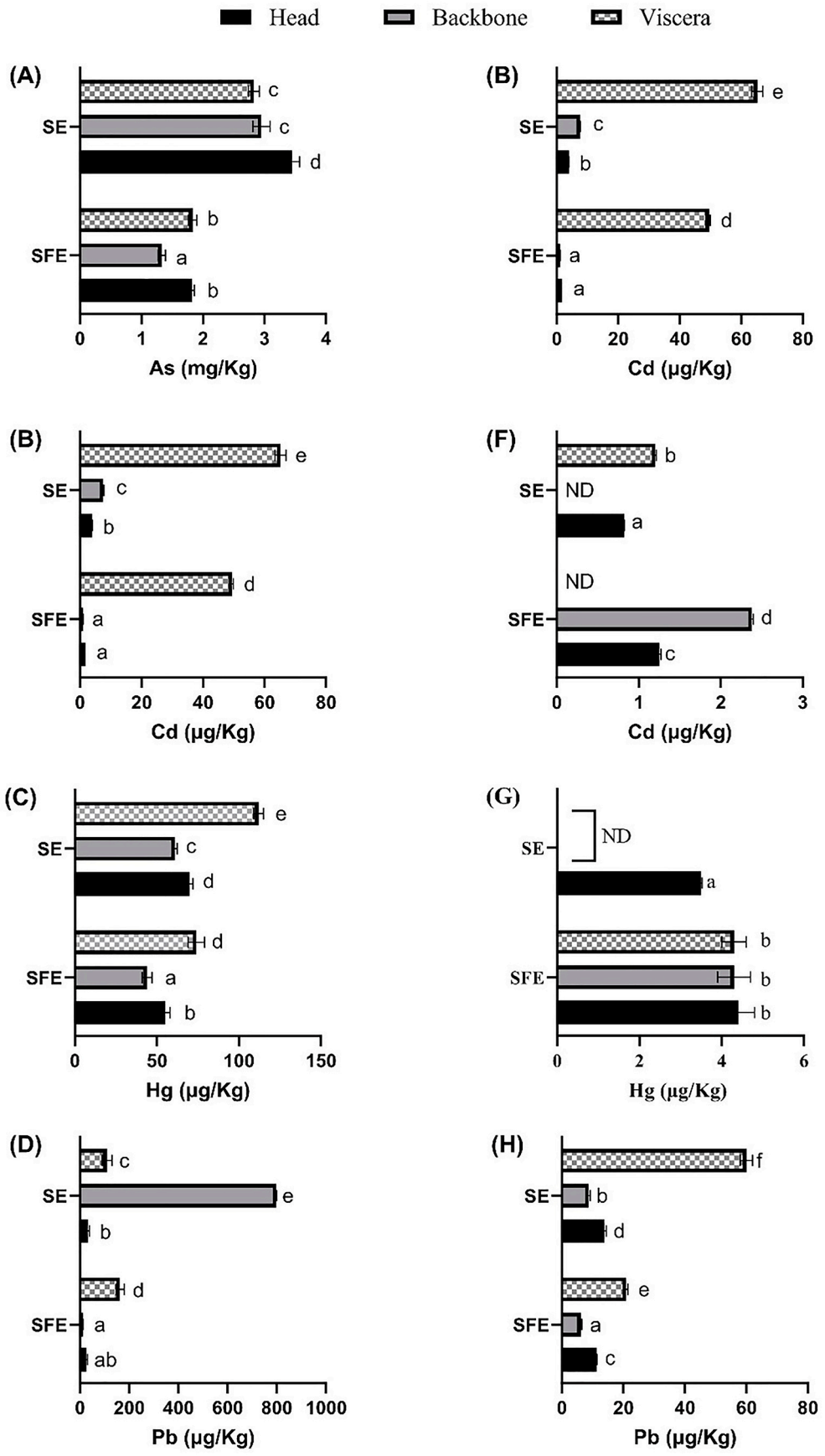

Fig. 3. Heavy metal content of solid matrices ((A), (B), (C), (D)) and liquid extracts ((E), (F), (F), (G)) obtained from salmon side streams using the supercritical fluid extraction (SFE) and solvent extraction (SE) methods.

and zinc (Zn) were also investigated in both the oil extracts and the remaining solid matrices obtained after SFE and SE methods. The results are presented in Figs. 4–5, revealing a notable difference in the mineral contents and profiles among the different salmon side streams (head, backbone, and viscera) studied, being the SFE-head and SFE-backbone solid matrices the highest source of Ca (49.7 mg/g and 24.2 mg/g, respectively), while SE viscera solid matrices were the highest source of Zn (791 mg/kg).

For the head, the SFE solid powder showed higher values for Mg, P, and Ca compared to the SE method, while K, Fe, and Zn contents were lower in the SFE solid matrices. Conversely, SFE oil extracts exhibited lower Mg, Ca, and Zn values compared to the SE method. Regarding the backbone, SFE solid matrices had higher Mg, P, and Ca contents than SE, while the opposite trend was observed for the oil extracts. In viscera solid matrices, SE showed the highest values for Mg (2.3 mg/kg), K (13.5 mg/kg), Fe (114.0 mg/kg), and Zn (791.0 mg/kg) compared to the other salmon side streams. Similarly, the oil extracts from SE viscera contained the highest mineral values (89.7 mg/kg for Mg, 566.0 mg/kg for P, 503.0 mg/kg for K, 144.0 mg/kg for Ca, 1.4 mg/kg for Fe, 12.7 mg/kg in Zn) across all six types. These results are in full agreement with a previous study which also found that the viscera side streams can contain large quantities of minerals (M. Wang et al., 2023).

SFE and SE employed as different oil extraction methods and for obtaining the remaining solid matrices from salmon side streams, revealed different mineral profiles and content. Figs. 4–5 illustrates that, compared to n-hexane extraction, SFE retained fewer minerals in oil extracts while preserving more mineral content in the remaining solid matrices, particularly in the backbone and viscera. This aligns with previous researches showing viscera side streams as rich sources of minerals (Pateiro et al., 2020). Although some elements were below detection limits or presented low concentrations, this research underscores the significant mineral potential in salmon side streams remaining solid matrices after using SFE and SE methods. It should also be noted that Mg, P, and K are more readily extracted with SFE, while

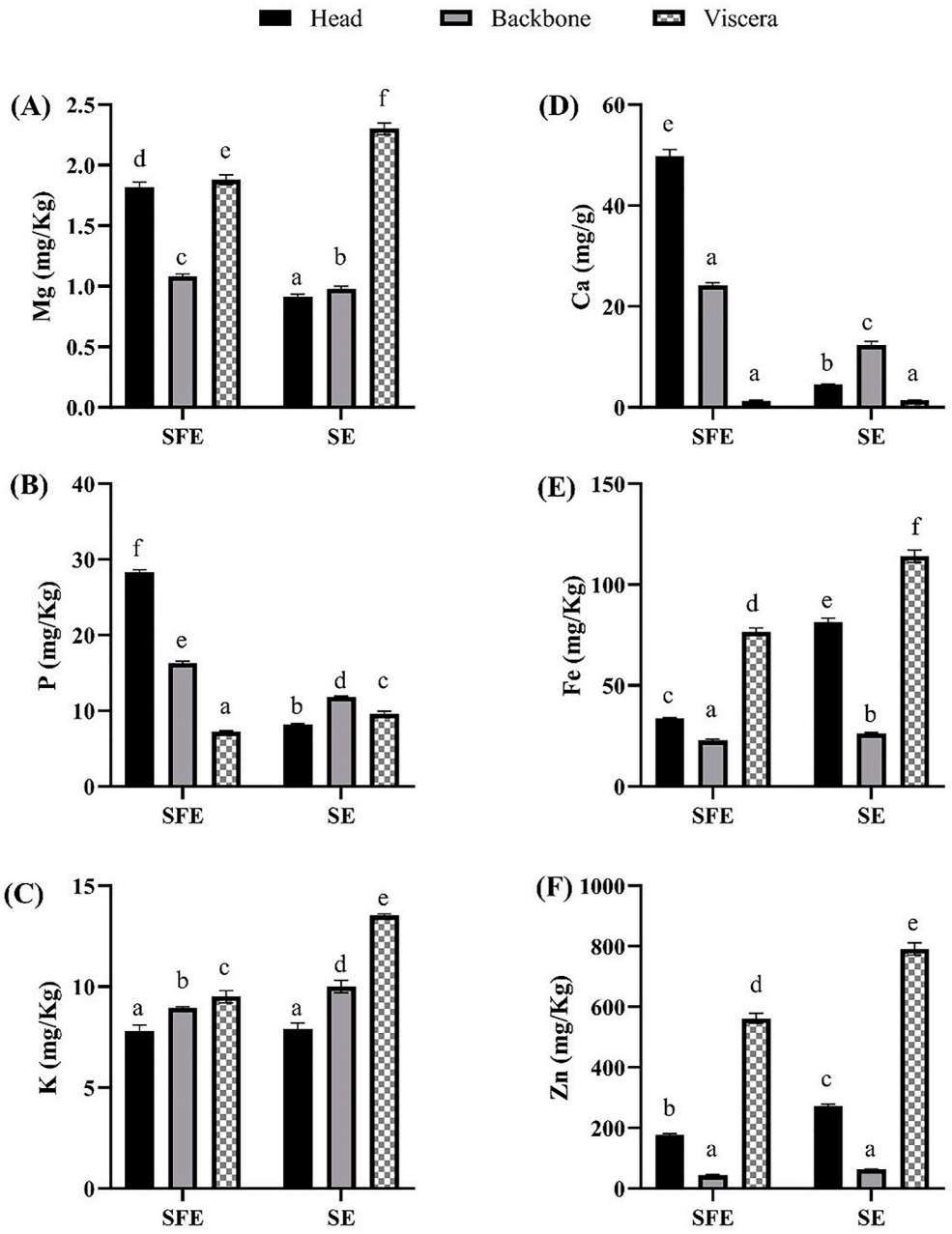

Fig. 4. The mineral content of solid matrices obtained from salmon side streams using the supercritical fluid extraction (SFE) and solvent extraction (SE) methods.

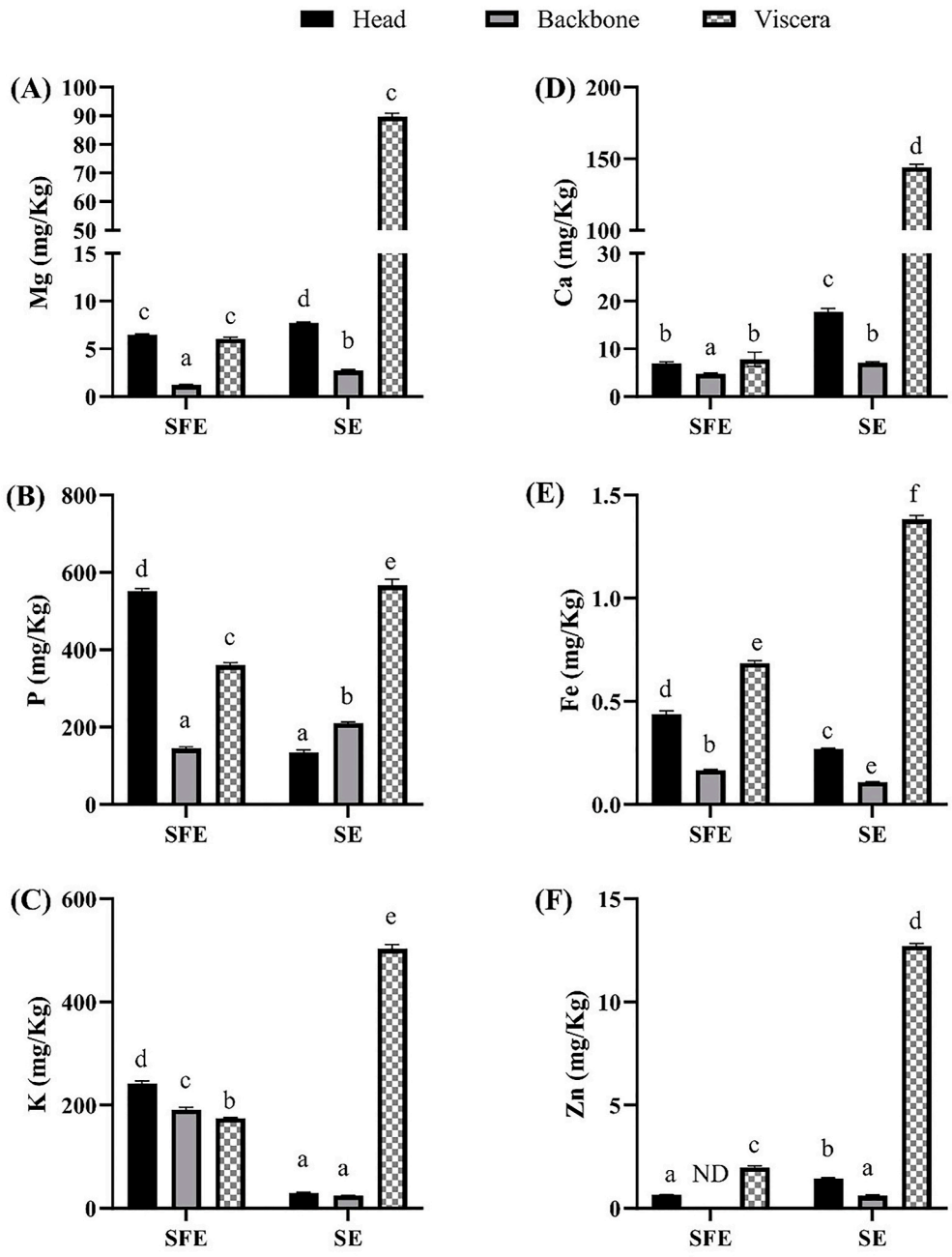

Fig. 5. Mineral content of liquid extracts obtained from salmon side streams using the supercritical fluid extraction (SFE) and solvent extraction (SE) methods.

Ca, Fe, and Zn are more efficiently extracted using *n*-hexane.

Although SFE has been widely used to recover oils, there are no consistent reports about the potential utilization of the remaining solid (cakes) obtained after the extraction. A previous study (Aas, Åsgård, & Ytrestøyl, 2022) reported the mineral profiles of the Atlantic salmon whole body, including calcium (Ca) (2849 mg/kg), potassium (K) (2741 mg/kg), phosphorous (P) (3137 mg/kg), iron (Fe) (13 mg/kg), and sodium (Na) (695 mg/kg), separately. Furthermore, the minerals contained in salmon are closely related to human health. For instance, Mg supports bone, nerve, and heart functions, and regulates blood pressure, and K helps distribute water in cells, maintains acid-base balance, and establishes membrane potential for nerve and muscle activity (Cilla et al., 2019; Nkansah, Korankye, Darko, Dodd, & Opoku, 2023; M. Wang et al., 2023). Considering the great amount of proteins and minerals inside the salmon side streams, and the growing search for alternative protein sources (Smith et al., 2024), this process seems a very useful tool to recover proteins and minerals (i.e, Ca, K, P, Fe, and selenium (Se)) with important beneficial effects on human health from marine side streams, especially considering the defatting process promoted by SFE itself, which is a first step prior recovering proteins from food and side streams.

3.5. Protein content

The protein content of solid matrices (cakes obtained after the SFE and SE) is shown in Fig. 6. From the results obtained, significant differences in the protein content between the salmon side streams subjected to SFE and SE were found. In the SFE -treated sample, the protein content in the remaining solid matrices was 66.0 g/100g for the head, 52.0 g/100g for the backbone, and 50.6 g/100g for the viscera. In contrast, when using SE, the protein content for the head was 71.2 g/100g, for the backbone was 69.6 g/100g, and for the viscera was 56.3 g/

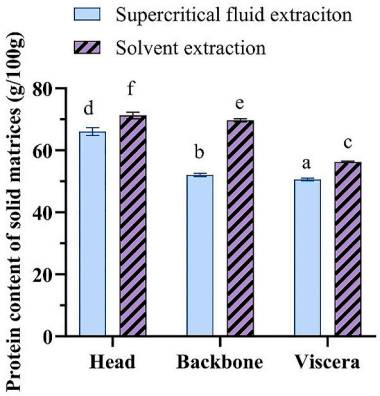

Fig. 6. Protein content of solid matrices obtained from salmon side streams using the supercritical fluid extraction (SFE) and solvent extraction (SE) methods.

100g. Notably, for the same salmon side stream tissue, SE with n-hexane as solvent exhibited a higher protein content profile in the remaining solid matrices. This fact can be attributed to the fact that SFE may accelerate lipid transport from the raw side stream tissue to the liquid extracts under the SFE treatment, thus reducing the protein content in the solid matrices after SFE.

3.6. Bioactive peptides (BPs)

Proteins from fish side stream have been identified as potential sources of bioactive peptides with diverse beneficial properties, including anti-inflammatory, antihypertensive, antimicrobial, antioxidative, anti-cancer, anti-obesity, and anti-hyperglycemia effects (Ucak et al., 2021; Yuan, Ye, Hou, & Chen, 2024). This study focuses specifically on investigating the presence of potential bioactive peptides in the remaining solid matrices obtained after the application of SFE or SE to salmon side streams (Fig. 7).

Six types of bioactive peptide activities, including antioxidative, anti-inflammatory, antithrombotic, immunomodulating, anticancer, and hypolipidemic properties, utilizing both SFE and SE methods, were evaluated and identified in our samples (Supplementary Tables 1–6). Especially, a few prominent peptide sequences were identified,

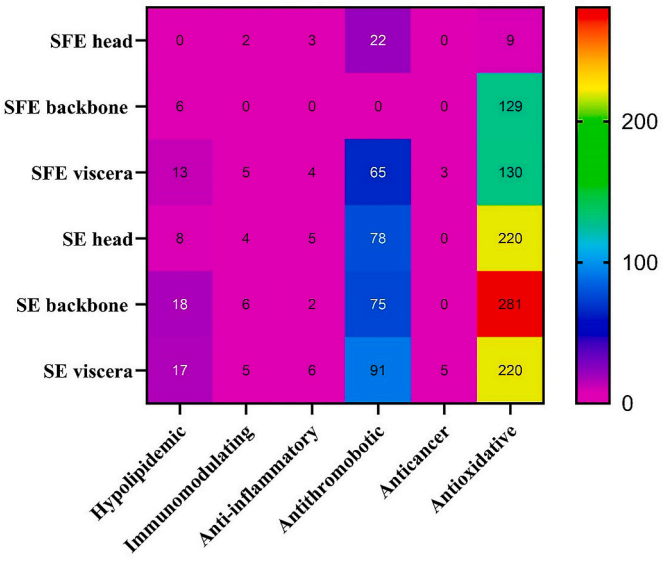

Fig. 7. The bioactive peptides activity of solid matrices obtained from salmon side streams utilizing the supercritical fluid extraction (SFE) and solvent extraction (SE) methods.

including antioxidant (EL), anti-inflammatory (GPAGPL), antithrombotic (GP, PGP), immunomodulating (YG), anticancer (VVV), and hypolipidemic (EF) Despite its efficiency, the use of n-hexane extraction raises concerns about sustainability and environmental impact, emphasizing the need for green and sustainable development practices. However, the *n*-hexane extraction with solid matrices retained more BPs than the SFE method; with the latter extraction approach demonstrating the highest efficiency and environmentally friendly characteristics, also avoiding the use of harmful solvents and extra solvent removal operations.

Fig. 7 illustrates bioactive peptides with potential antioxidant properties found in salmon side streams (head, backbone, and viscera), with the highest amount of peptides observed in SE backbone (281), followed by SE viscera (220) and SE head (220). In contrast, the remaining solid matrices obtained after using the SFE method did not exhibit a high antioxidant ability. The number of potential bioactive peptides in the solid matrices obtained after SFE was 9 for the head, 129 for the backbone, and 130 for the viscera.

Similarly, eighteen peptides were purified from the Monkfish (*Lophius litulon*) swim bladders by Sheng et al. using ultrafiltration and gel permeation chromatograph methods. The isolated peptides presented remarkable antioxidant activity, including the $O_2^{\cdot}$ scavenging capacity, $ABTS^{+\cdot}$ scavenging ability, and ability of lipid peroxidation inhibition (Sheng et al., 2023).

Inflammation is the reactivity to harmful stimuli, reward processing, somatic symptoms, and uncontrolled inflammatory mediators associated with some diseases (Dooley et al., 2018). In this sense, the novel anti-inflammatory KIWHHTF, VHYAGTVDY, and HLDDALRGQE peptides, derived from the sturgeon muscle, were identified by Gao et al., and the anti-inflammatory mechanism for these peptides was observed as a consequence of the suppression of the MAPK pathway (Gao et al., 2020).

Antithrombotic peptides derived from food have gained attention due to their high biological activity, low toxicity, and easy metabolism within the human body, performed as the potential ingredients in the health-promoting functional food targeting thrombus (Cheng, Tu, Liu, Zhao, & Du, 2019). Previous researchers revealed that bioactive peptides are considered beneficial to promote various beneficial effects. Typically, bioactive peptides are encrypted and kept inactive within the food protein primary structure(Marcone, Belton, & Fitzgerald, 2017). The prior research indicated that bioactive peptides usually contain 3-20 amino acid residues (X. Wang, Yu, Xing, & Li, 2017). In this study, the antithrombotic activity has been detected from our salmon side streams, and the potential antithrombotic peptides amount for SFE head, SFE backbone, SFE viscera, SE head, SE backbone, and SE viscera were 22, 17, 65, 78, 75, and 91, respectively. Incorporating antithrombotic peptides into the diet offers a convenient and effective means of preventing and mitigating cardiovascular diseases (CVDs) (Cheng et al., 2019).

The egg yolk lipoproteins were hydrolyzed using trypsin, and the bioactivity of the resulting peptides was assessed by an in silico analysis in recent research; the result shows that KVQWGIIPSWIKK was the most promising antihypertensive peptide found in the permeates (Marcet, Delgado, Díaz, Rendueles, & Díaz, 2022). Furthermore, this study identified the anti-inflammatory, immunomodulating, anticancer, and hypolipidemic bioactive activities in salmon head, backbone, and viscera using both SFE and SE. Recent research supports the notion that various bioactive peptides with biological activity can be obtained from the diet, encompassing anticancer (Ali Redha, Valizadenia, Siddiqui, & Maqsood, 2022), anti-inflammatory (Cruz Chamorro et al., 2022), antioxidants, anti-hypertensive (Daroit & Brandelli, 2021), antimicrobial (Zaky, Simal Gandara, Eun, Shim, & Abd El-Aty, 2022), and mineral binding functions (Sun et al., 2020). For instance, over the last years, some researchers have been focused on the isolation of BAPs with antioxidant, anti-inflammatory, angiotensin-converting enzyme (ACE) inhibitory, and α-glucosidase inhibitory properties from fermented

foods, such as lactic fermented camel milk (Dharmisthaben et al., 2021), fermented cucumbers (Fideler, Johanningsmeier, Ekelöf, & Muddiman, 2019), and fermented rubbing cheese (Wei et al., 2022). Similarly, some researchers have summarized the literature on BPs in fermented food, focusing on the isolation, purification, and identification of the properties in fermented foods-derived BAPs, which provides the foundation and future direction for the application of fermented food-derived BPs in the food and the pharmaceutical fields (Guo, Chen, & Chen, 2023).

3.7. Analysis of ABTS $^{+}$ free radical cation scavenging capacity

The Trolox equivalent antioxidant capacity (TEAC) of liquid extractions using both SFE and SE methods was assessed in this study. Supplementary Fig. 1 reveals a superior antioxidant activity in the head and backbone extracted via SFE compared to the conventional extraction method.

In addition, Supplementary Fig. 1 further illustrates that TEAC values for liquid extracts processed through SE did not show any significant difference between the head (78.4 μM Trolox), backbone (90.1 μM Trolox), and viscera (83.6 μM Trolox). However, there is a significant difference observed between SFE-extracted viscera and the other salmon side streams, with viscera exhibiting the highest antioxidant capacity (91.2 μM Trolox).

The SFE method demonstrated a superior TEAC ability compared to SE liquid extracts in this study. Remarkably, SE-viscera also exhibited excellent TEAC ability relative to the salmon head and backbone extracted using SFE. While n-hexane is commonly used as an extraction solvent due to its efficiency in extracting lipid components, its harmful effects on human health need careful removal from edible food. On the other hand, SFE has gained attention in the food processing industry due to its environmental friendliness, use of green solvents, and enhanced safety for edible products (Pagano, Campone, Celano, Piccinelli, & Rastrelli, 2021). Researchers have identified various mechanisms through which phenolic antioxidants operate, including single electron transfer, hydrogen atom transfer, transition metal chelation, and sequential proton loss electron transfer (Zeb, 2020). Similarly, other studies have concluded that several pathways can effectively reduce excess free radicals, whether directly or indirectly (Guo et al., 2023; Tonolo et al., 2020; Zhou et al., 2020).

Overall, the SFE method presents better antioxidant activity in TEAC for salmon head and backbone. Considering concerns related to toxicity, solvent effects, and environmental pollution in the food industry, SFE emerges as a forward-looking solution addressing the main challenges and opportunities associated with fish side streams processing.

4. Conclusions

In conclusion, the SFE and SE methods were adapted to extract oil and recover potentially useable remaining solid matrices from salmon side streams. Our research shows that both the oil and solid matrices have significant potential. They are rich in essential fatty acids and can be used in the production of high-added-value compounds. These include bioactive peptides (BPs), minerals, and antioxidants.

A clear difference in the amount of BPs with different functional properties was found between the remaining solids obtained by SFE and SE. Six types were identified: antioxidant (EL), anti-inflammatory (GPAGPL), antithrombotic (GP, PGP), immunomodulating (YG), anticancer (VVV), and hypolipidemic (EF). Remarkably, potential antioxidant peptides were obtained (based on the BIOPEP-UMP database). The ICP-MS analysis indicated elevated heavy metal levels in the SFE oil extracts from salmon head and backbone, contrasting with the reduced concentrations found in the remaining solid matrices, opening a door to the potential use of remaining solid matrices obtained after SFE. Conversely, salmon viscera showed a reversed pattern. Despite these variations, all mineral and heavy metal levels remained within safe limits. Liquid extractions from both extraction methods (SFE and SE) exhibited promising antioxidant activities via the ABTS assay. Overall, our results indicate that salmon side streams solids and oil extracts are nutrient and bioactive-rich, showing their potential to be exploited.

CRediT authorship contribution statement

Yixuan Liu: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation. **Houda Berrada:** Writing – review & editing, Supervision, Resources, Investigation. **Noelia Pallarés:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Francisco J. Marti-Quijal:** Writing – review & editing, Formal analysis, Data curation. **Juan Manuel Castagnini:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Francisco J. Barba:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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.lwt.2024.117116.

Data availability

Data will be made available on request.

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