Food and Bioprocess Technology (2025) 18:1873–1892
https://doi.org/10.1007/s11947-024-03553-3

RESEARCH

Is Pulsed Electric Field (PEF) a Useful Tool for the Valorization of Solid and Liquid Sea Bass Side Streams?: Evaluation of Nutrients and Contaminants

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Received: 5 May 2024 / Accepted: 6 August 2024 / Published online: 27 August 2024

Abstract

In this study, fresh sea bass's (*Dicentrarchus labrax*) heads, skin, viscera, and muscle were evaluated for their potential valorization as a source of nutrients and bioactive compounds. For this purpose, a pulsed electric field (PEF) treatment (1.0 kV/cm field strength and 220.5 kJ/kg specific energy for head, 3.0 kV/cm and 299.4 kJ/kg for skin, 3.0 kV/cm and 123.7 kJ/kg for viscera and muscle) was used. Subsequently, extraction processes were carried out using either 100% water or 50% ethanol. Protein assessment (protein content and bioactive peptides' identification), as well as ICP-MS analysis of minerals and heavy metals, was conducted on both the solid matrices and liquid extracts. The findings indicate that the choice of solvent (100% water or 50% ethanol) and PEF treatment significantly ($p < 0.05$) affected protein recovery in the sea bass side streams liquid extracts, while a considerable protein amount was retained in the solid matrices. Furthermore, the ICP-MS analysis of minerals revealed that PEF treatment significantly ($p < 0.05$) improved mineral recovery in the head and muscle liquid extracts. However, a considerable amount of minerals remained in the solid matrices. Lower contents of heavy metals were found in the liquid extracts compared to the solid matrices, being anyway the quantities of the five heavy metals analyzed within edible and safe limits. Additionally, the total antioxidant capacity (TAC) of sea bass side stream extracts was assessed to measure the potential antioxidant bioactive compounds in the liquid extracts. PEF treatments significantly ($p < 0.05$) increased the TAC of the liquid extracts from sea bass viscera, as opposed to other side streams. Both 100% water and 50% ethanol were effective as extraction solvents, promoting the recovery of high-added-value compounds not only from the liquid extracts but also from solid matrices. Thus, PEF pre-treatment can be considered a valuable technique to enhance fish side stream valorization.

Keywords PEF · Sea bass side streams · Bioactive compounds · ICP-MS · Protein · Solid matrices and liquid extracts

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Abbreviations

PEF	Pulsed electric field
TAC	Total antioxidant capacity
ICP-MS	Inductively coupled plasma spectrometer detector
TEAC	Trolox equivalent antioxidant capacity
ORAC	Oxygen radical absorbance capacity
Ca	Calcium
P	Phosphorus
Mg	Magnesium
k	Potassium
Fe	Iron
Zn	Zinc
Cu	Copper
As	Arsenic
Cd	Cobalt

Hg	Mercury
Pb	Lead

Abbreviations in Peptides' Sequence

A	Alanine
C	Cysteine
D	Aspartic acid
E	Glutamic acid
F	Phenylalanine
G	Glycine
H	Histidine
I	Isoleucine
K	Lysine
L	Leucine
M	Methionine
N	Asparagine
P	Proline
Q	Glutamine
R	Arginine
S	Serine
T	Threonine
V	Valine
W	Tryptophan
Y	Tyrosine

Introduction

A gradual increase in fish consumption has been observed over the past decades (FAO, 2022). This trend is also reflected in the growing increase of fish side streams produced by the food industry. The fish processing industry (FPI) is characterized by the significant production of seafood and marine products, whereas only 40% is used for human consumption, and more than 60%, mainly heads, skin, viscera, backbones, fins, roe, trimmings, etc., are discarded (Ideia et al., 2020; Zamora Sillero et al., 2018). Previous research determined that the non-consumption of fish side streams can be attributed to two primary factors. Firstly, fish side streams such as the viscera and blood are prone to rapid decomposition unless they are adequately processed or stored under favorable conditions (Chalamaiah et al., 2012; Rustad et al., 2011). Secondly, certain parts of fish side streams consist of more durable components, including bones, heads, scales, and skin (Saleh et al., 2022). Interestingly, fish side streams contain a considerable number of high-added-value compounds (nutrients and bioactive compounds), which can be explored and extracted for further application (Bruno et al., 2019; Seyedi et al., 2021; Vázquez et al., 2019). Sea bass, primarily found and farmed in the Mediterranean Sea and its adjacent waters (Munekata et al., 2020), is renowned for its delectable flesh, boasting high nutritional value and low-fat content (Yu & Xie, 2023). Proteins extracted from fish muscle contain numerous peptides showcasing various health benefits, such as antihypertensive, antithrombotic, immune-modulatory, and antioxidative properties (Ucak et al., 2021). The derived fish proteins boast a well-balanced amino acid composition, surpassing other animal protein sources (Caruso et al., 2020a).

Our team and other research groups have explored the application of liquid extracts obtained by different technologies, including pulsed electric fields (PEF) (Franco et al., 2020; Gómez et al., 2019; Gulzar & Benjakul, 2019; He et al., 2014, 2019; Kokkali et al., 2020), ultrasound (Munekata et al., 2021), accelerated solvent extraction (ASE) (Wang et al., 2021a), microwave extraction (Viji et al., 2022), and supercritical fluid extraction (SFE) (Kuvendziev et al., 2018) from fish side streams as a source of high-added-value compounds with biological properties, obtaining promising results. However, these studies typically overlooked the utilization of the residual solid portion, often focusing solely on the supernatant for subsequent analysis and discarding the solid residue. This practice poses a challenge in terms of efficiently utilizing side streams and promoting circular economy principles. Moreover, the inherent limitations associated with the direct use of fish side streams include factors such as limited digestibility, reduced consumer acceptability, inadequate handling and storage practices leading to rapid fish quality deterioration, the accumulation of toxic metals in tissues (Banan et al., 2022; Kazemi et al., 2022a; Selvakumar et al., 2021), and the potential presence of contaminants (Saravanan et al., 2023; Thirukumaran et al., 2022). In addition, some problems in the fish side stream treatment processing will appear including poor mechanical function, increased unpleasant smell, and increased production costs (Fauconnet et al., 2019; Saravanan et al., 2023).

Considering this scenario, the application of PEF technology holds the potential to significantly overcome this limitation when it comes to the valorization of fish side streams. It is worth noting that to the best of our knowledge, there are no existing reports on the application of PEF techniques for the valorization of sea bass side streams, with the aim of exploring the nutritional and bioactive components within solid matrices and liquid extracts. Consequently, the application of PEF presents a dual opportunity for repurposing these side streams, not only by extracting compounds from the liquid extracts but also by considering the potential reuse of the solid matrices. To achieve this, a comprehensive evaluation is essential, encompassing both the presence of contaminants and the retention of nutrients in both the solid matrices and liquid extracts.

In this study, we employed PEF as a pre-treatment technology in conjunction with extraction solvents such as 100% water and 50% ethanol. Our objective is to extract high-added-value compounds from sea bass side streams,

yielding both solid matrices and liquid extracts. Additionally, we evaluated the protein, bioactive peptides, mineral, and heavy metal content and profile of both the remaining solid matrices and liquid extracts. This research seeks to offer a comprehensive understanding of the potential utilization of solid matrices and liquid extracts from sea bass side streams, obtained through PEF pre-treatment while addressing the challenges associated with extracting valuable components from fish side streams.

Materials and Methods

Samples

Fresh slaughtered sea bass was purchased from the local supermarket (Valencia, Spain). Side stream fractions, including head (4.8% protein content (w/w, total weight)), muscle (9.9% protein content), skin (2.1% protein content), and viscera (8.0% protein content), were manually obtained from the sea bass.

Chemicals and Reagents

Trolox (6-hydroxy-2,5,7,8-ttetramethyl-chroman-2-carboxylic acid), fluorescein sodium salt, AAPH (2,2′-azobis-2-methyl-propanimidamide), ABTS (2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonic acid)), potassium persulfate ($K_2S_2O_8$), sodium phosphate dibasic (Na_2HPO_4), and potassium dihydrogen (KH_2PO_4) were purchased from Sigma-Aldrich (Steinheim, Baden-Württemberg, Germany); deionized water was obtained in the Milli-Q SP reagent water system (Millipore Corporation, Bedford, MA, USA).

PEF Treatment, the Extraction Process, Solid Matrices, and Liquid Extracts Obtained

The head, skin, viscera, and muscle collected from the fresh sea bass were processed by a PEF-Cellcrack III (German Institute of Food Technologies (DIL)) equipment (ELEA, Quakenbrück, Osnabrück, Germany) located at the laboratory of ALISOST team at the Faculty of Pharmacy and Food Sciences, Universitat de València (València, Spain), to carry out the experiments. The weight ratio of sample to deionized water was 100.0 g:1500.0 g for the head and 45.0 g:675.0 g for the skin/viscera/muscle. The optimal PEF treatment conditions (1.0 kV/cm field strength and 220.5 kJ/kg specific energy for head with a 12-L treatment chamber, 3.0 kV/cm and 299.4 kJ/kg for skin, 3.0 kV/cm and 123.7 kJ/kg for viscera and muscle with a 900-mL treatment chamber), processing chamber, and the amount of added water were obtained from our previous works (Martí-Quijal et al., 2023). The magnetic stirrer time at room temperature for each sea bass side stream varied. Centrifugation was performed at 4000 × rpm, at 4 °C for 15 min. The supernatant (liquid extracts) and solid remaining sample (solid matrices) were separated via filtration (filter paper). PEF treatment and extraction processing are presented in Figs. 1 and 2.

Protein Recovery

The protein contents of liquid extracts and solid matrices were determined according to the Dumas method (Jimenez & Ladha, 1993; Serrano et al., 2013) with a Flashsmart Elemental Analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA). All the samples were freeze-dried and ground to a powder prior to analysis. Dried sample powders of 0.1 g (weighed with an accuracy of 0.0001 g) were wrapped in tin foil, placed in the sample pan, then fully burned in

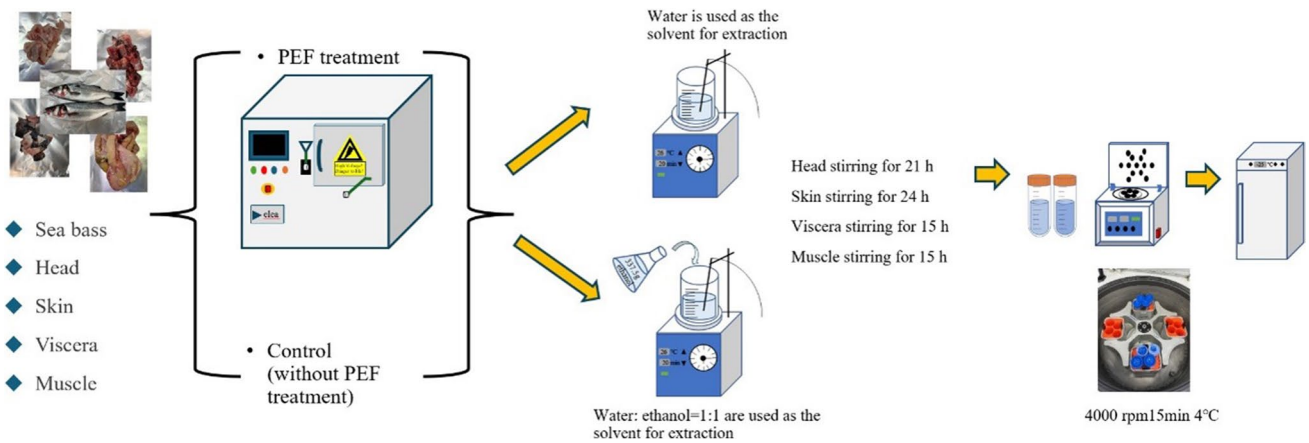

Fig. 1 Pulsed electric field (PEF) treatment and extraction processes followed for the recovery of high-added-value compounds from sea bass side streams

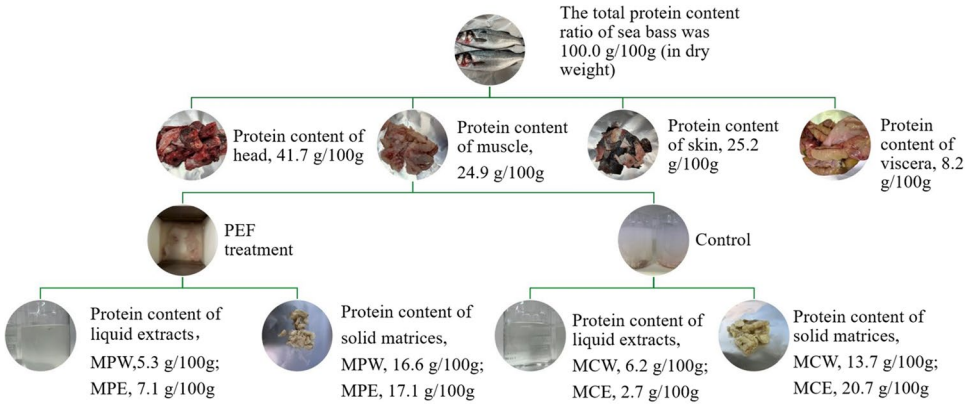

Fig. 2 Protein content ratio (in dry weight) changes during the extraction process. MCW, muscle control-100% water; MPW, muscle PEF-100% water; MCE, muscle control-50% ethanol; MPE, muscle PEF-50% ethanol

high-purity oxygen (≥ 99.99%) in a combustion reactor (900–1200 °C), and transported to the reduction furnace (800 °C) using helium as carrier gas. Nitrogen amounts were detected after reduction and quantified using the following formula, and the nitrogen-to-protein conversion factor is 6.25 for fish and fish side streams (de la Fuente et al., 2021):

$$\text{Protein}\ (\%) = 6.25 \times \text{Nitrogen}\ (\%) \quad (1)$$

$$\text{The protein content ratio} = \frac{\text{protein of fish tissue used}}{\text{total prote in of l fish used}} \times 100\% \quad (2)$$

Identification of Bioactive Peptides

The bioactive peptide identification process in the liquid extracts adhered to the established methodology described in a previous study (de la Fuente et al., 2021), employing liquid chromatography-tandem mass spectrometry (LC–MS/MS). Soluble peptides were isolated and subsequently analyzed using a nano ESI qTOF MS (6600plus TripleTOF, ABSCIEX, Framingham, MA, USA) equipped with a trap column (ChromXP C18, 3 m 120 Å, 350 m, 0.5 mm; Eksigent). The peptide identification process was carried out through the ProteinPilot v5.0 search engine, developed by AB SCIEX, coupled with LC–MS/MS analysis. Subsequently, the identified antithrombotic and oxidative peptides were cross-referenced against the BIOPEP-UWM database, serving as the reference database for this study (Minkiewicz et al., 2019).

ICP-MS Determination of Minerals and Heavy Metals

Mineral and heavy metal analysis was conducted by a microwave accelerated reaction system (MARS, CEM, Vertex, Spain) and an inductively coupled plasma spectrometer detector (ICP-MS, Agilent model 7900, Santa Clara, CA, USA). The MARS was utilized for the acid mineralization of the sea bass side stream fractions. 0.2–0.4 g sample, 1 mL H_2O_2 (30%, v/v), and 4 mL HNO_3 (64%, v/v) were added into the Teflon vessel, and then digested with MARS at 800 W and 180 °C for 15 min. After the digested sample was cooled and the nitrogen was removed, filtered through filter paper, and brought up to the desired volume with distilled water. The identification and quantity of minerals and heavy metals were conducted by the ICP-MS. The calibration curve with an external standard setting was used to determine the mineral and heavy metal contents, and the results were expressed as mg/kg or μg/kg (Wang et al., 2023).

Antioxidant Capacity

The Trolox equivalent antioxidant capacity (TEAC) assay was performed in the liquid extracts according to a previously described method (Wang et al., 2021b; de la Fuente et al., 2021). The mother solution was obtained by mixing 25 mL of 7 mM ABTS and 440 μL of 140 mM potassium persulfate solution and stored in the dark at room temperature for 12–16 h before use. The mother solution was then diluted with ethanol until its absorbance value reached 0.70 ± 0.02 under a 30 °C. 100 μL of sample/standard and 2.5 mL of the working solution were mixed, and then, the absorbance was measured at 734 nm after 10 min in darkness in triplicate samples. The absorbance values for the working solution and the sample are A_0 and A_f. The percentage of absorbance inhibition was calculated and compared to the Trolox standard curve to obtain the results as μM Trolox equivalent as follows: $1-(A_f/A_0)\times 100$. The standard curve equation is $Y = 0.1718 \times X + 1.6779$, $R^2 = 0.999$. The results were presented as μM Trolox equivalents.

The oxygen radical absorbance capacity (ORAC) assay was also performed in the extracts using the fluorimetric method described by Wang et al. (2021b) and de la Fuente et al. (2021). Briefly, 50 μL Trolox (1 mM), diluted sample, or phosphate buffer (blank), and 50 μL fluorescein were added to 96-well plates. The plates were incubated at 37 °C for 10 min, and 25 μL AAPH (221.25 mM) was then added in each well. The absorbance value of the samples

was measured at 520 nm in a FLUOstar OMEGA plate reader (BMG Labtech GmbH, Ortenberg, Germany) with fluorescence filters for excitation at 485 nm and an emission wavelength of 535 nm. All tests were performed in triplicate. The difference in the area under the fluorescence decay curve (AUC) between the blank and the sample over time was taken into account when calculating the results. The calculation formula used was:

$$\text{ORAC (Trolox)} = \frac{A_{\text{Sample}} - A_{\text{Blank}}}{A_{\text{Trolox}} - A_{\text{Blank}}} \quad (3)$$

Statistical Analysis

All analytical tests were performed in triplicate, and the results are presented as average values. Statistical analyses were carried out using a three-way analysis of variance (ANOVA) followed by Duncan's multiple range test, with statistically significant differences considered at $p < 0.05$. This analysis was conducted utilizing SPSS 20.0 software (IBM Corp., Armonk, NY, USA) and data visualization was performed using GraphPad Prism (v.10.0.1 GraphPad Software Company, La Jolla, CA, USA). Additionally, GraphPad Prism was employed for creating graphical representations.

Results and Discussion

Protein Mass Balance

The protein mass balance (in dry weight) from differentially processing sea bass side streams (head, skin, viscera, and muscle) is illustrated in Fig. 3. As can be seen in the figure, the application of PEF technology significantly enhanced protein recovery in the liquid extracts, particularly when 50% ethanol was employed as the extraction solvent.

When it comes to the sea bass heads, the results revealed that the three primary sources of variation for protein content in the end samples, namely the end product fraction (solid matrices or liquid extracts), the interaction state × treatment (PEF or without PEF), and solvent (100% water or 50% ethanol), accounted for 74.7%, 16.7%, and 4.6% of the total variation, respectively. In the case of the sea bass skins, the factors contributing to the total variation were fraction, fraction × treatment, and fraction × solvent, explaining 73.2%, 8.3%, and 8.0% of the total variance, respectively.

As for the muscle of sea bass, the results revealed that the three primary sources of variation, namely fraction, fraction × treatment × solvent, and fraction × solvent, accounted for 88.4%, 5.6%, and 3.4% of the total variation, respectively.

In sea bass heads, skin, and muscle materials, the control-50% ethanol-solid and PEF-50% ethanol-solid treatments yielded the lowest protein recovery levels, or else higher protein levels remaining in the solid matrix fraction (37.5 g/100 g for head, 20.3 g/100 g for skin, 8.8 g/100 g for viscera, 20.7 g/100 g for muscle and 25.9 g/100 g for head, 20.2 g/100 g for skin, 7.7 g/100 g for viscera, 17.1 g/100 g for muscle, respectively). The highest protein content ratio in the liquid extracts from heads, skin, and muscle was found after following PEF processing, representing the protein content ratio in dry weight (19.8 g/100 g, 8.5 g/100 g, and 7.1 g/100 g, respectively).

The primary factor significantly influencing protein content in solid matrices and liquid extracts was the fraction (solid or liquid fraction), demonstrating that still a large number of proteins remained in the solid matrices, as clearly depicted in Fig. 3. According to the results of the three-way ANOVA, the fraction × treatment (combined effects of state and treatment method) represented the second

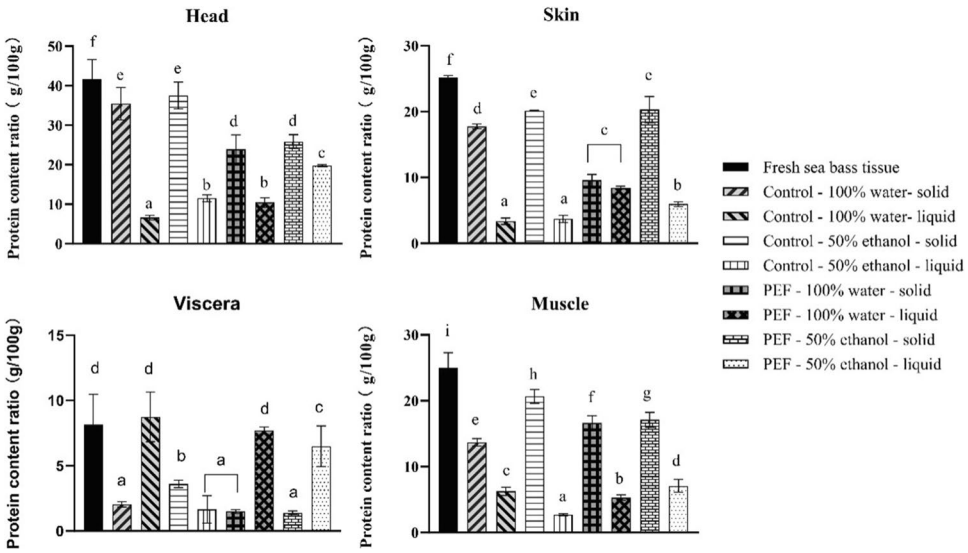

Fig. 3 Protein content ratio of fresh sea bass side streams (head, skin, viscera, and muscle), liquid extracts, and solid matrices (The protein content ratio = ((protein of fish tissue used) / (total protein of l fish used)) × 100%). Significant differences between different data are represented by different letters, starting with the smallest value marked with a

most significant factor for the head and skin. Additionally, a notable protein recovery enhancement was observed for heads, skin, and muscle liquid extracts when 50% ethanol was employed as the extraction solvent. Recent findings supported that PEF treatment significantly enhances the protein recovery in liquid extracts from the sole head and skin, potentially attributed to the "electroporation" phenomenon, which facilitates protein dissolution within fish side streams (Wang et al., 2021a). Similarly, recent research has also demonstrated an increase in protein recovery in liquid extracts following PEF pre-treatment (Martí-Quijal et al., 2023).

In the sea bass viscera, the three primary sources of variation regarding the protein recovery results, namely the fraction, fraction × treatment × solvent, accounted for 46.9%, 10.5%, and 8.9% of the total variation, respectively. As depicted in Fig. 3 for the viscera, the highest protein content was analyzed in the liquid fractions, with the highest protein content ratio in the extract (8.8 g/100 g) achieved in control-100% water-liquid.

The choice of solvent (100% water or 50% ethanol) notably affected protein recovery in the liquid extracts. Equally significant was the role played by PEF treatment in the extraction process. Innovative and environmentally friendly protein extraction methods, such as PEF (Jaeschke et al., 2019) and UAE (Gadalkar & Rathod, 2020), offer distinct advantages, including higher purity, reduced energy consumption, and minimized effluent generation when compared to conventional techniques (Chemat et al., 2019). In our study, both the solvent (100% water or 50% ethanol) and PEF treatment effectively promoted protein recovery in liquid extracts obtained from sea bass side streams. Notably, the solid matrices still contained a substantial protein amount, suggesting their potential as a valuable source of healthy protein, and the potential protein bioactivity would be followed by investigating the identification of bioactive peptides. Nevertheless, another limitation with PEF in this study was not having considered the effect of PEF on the extraction of lipids compounds because 100% water or 50% ethanol was used as the extraction solvent. Previous researcher has concluded that the application of PEF treatment before oil extraction can lead to lesser extraction time and higher extraction yield owing to the selective portion of oil-bearing cells from the high throughput of PEF (Naliyadhara et al., 2022).

Identification of Bioactive Peptides (Antithrombotic and Antioxidative Activity)

Previous studies have classified bioactive peptides into endogenous and exogenous types, with endogenous peptides synthesized within an organism through enzymatic processes (Okoye et al., 2022), and exogenous peptides obtained from diet, supplements, and additives (Chakrabarti et al., 2018). In this research, three samples (muscle PEF-50% ethanol, muscle PEF-100% water, and muscle control-100% water) were selected for further investigation based on protein content. Sea bass side streams' bioactive peptides were explored using LC–MS/MS, revealing antioxidant and antithrombotic activities.

The antithrombotic activity of bioactive peptides extracted from sea bass side streams' solid matrices with 100% water or 50% ethanol is presented in Table 1. Notably, only one peptide sequence (DRGDSGAAGPAGPAGPAGPRGPA) exhibiting antithrombotic properties was identified in muscle PEF-50% ethanol extract. This particular peptide demonstrated seven distinct activity sequences (GPRG, GPRGP, GPRGPA, GPR, RGDS, GP, and RGD). In contrast, muscle PEF-100% water and muscle control-100% water extracts yielded eight and five peptide sequences, respectively, each characterized by a single short sequence of activity involving specific amino acids. Previous research reveals that antithrombotic peptides play a crucial role in inhibiting fibrinogen binding to a specific receptor region on the platelet surface and also impede platelet aggregation (Sabbione et al., 2016).

Regarding antioxidant activity, the number of peptide sequences from muscle PEF-50% ethanol, muscle PEF-100% water, and muscle control-100% water extracts were 5, 55, and 105, respectively. As it can be seen in Table 2, from the muscle PEF-100% water extract were identified more peptide sequences compared to the muscle PEF-50% ethanol extract. Regarding the effect of the extraction solvent on peptides, it has been seen that 100% water sea bass side stream extracts contained more peptides with antioxidant activity. Previous findings in the available literature indicate that the potential antioxidant activity of the peptides evaluated was due to the presence of specific amino acid glycopeptide sequences' aromatic rings (Martí-Quijal et al., 2023; Zou et al., 2016). Moreover, previous researchers isolated collagen from redlip croaker (*Pseudosciaena polyactis*) scales, resulting in six cytoprotective peptides with strong antioxidant abilities (including radical-scavenging activities, lipid peroxidation-inhibiting ability, and DNA damage-protective activities against oxidation), suggesting the results that three peptides (GPEGPMGLE, EGPFGPEG, and GFIGPTE) might serve as potential antioxidants applied in nutraceutical and pharmaceutical products (Wang et al., 2020). Similarly, eight collagen peptides from Spanish mackerel skin exhibited dose-dependent scavenging of free radicals and lipid peroxidation (Zhang et al., 2019).

Earlier studies have identified multifunctional properties of fish bioactive peptides, such as hypoglycemic, hypolipidemic, antihypertensive, and cytoprotective effects, from peptides such as LILPKHSDAD, LTFPGSAED (Zanoni et al., 2017), YDFYPSSTKDQQS, and

Table 1 Antithrombotic activity of bioactive peptides obtained from the sea bass side streams' solid matrices extracted with 100% water or 50% ethanol (only peptides' sequences with more than 80% of confidence were selected)

Solid matrices	Sequence	ID	Name of peptide	Involved amino acid	Location
Muscle PEF-50% ethanol	DRGDSGAAGPAGPAGPAG-PRGPA	3040	Inhibitor of fibrinogen and thrombin coagulation	GPRG	[18–21]
		3041	Inhibitor of fibrinogen and thrombin coagulation	GPRGP	[18–22]
		3042	Inhibitor of fibrinogen and thrombin coagulation	GPRGPA	[18–23]
		3047	Antithrombotic peptide	GPR	[18–20]
		3126	Fibronectin inhibitor	RGDS	[2–5]
		3283	Antithrombotic peptide	GP	[9–10], [12–13], [15–16], [18–19], [21–22]
		9660	Antithrombotic peptide	RGD	[2–4]
Muscle PEF-100% water	WISKQEYDEAGPSIVHR	3283	Antithrombotic peptide	GP	[11–12]
	ISKQEYDEAGPSIVHRKC	3283	Antithrombotic peptide	GP	[10–11]
	N(Deamidated)LKPGELPT-CESLKDTIAR	3285	Antithrombotic peptide	PG	[16–17]
	FSVDEEFPDLSLHNNHMA	3354	Antithrombotic peptide	DEE	[4–6]
	WISKQEYDEAGPSIVHRKC	3283	Antithrombotic peptide	GP	[11–12]
	FSVDEEFPDLSLHNNHM	3354	Antithrombotic peptide	DEE	[4–6]
	FSVDEEFPDLSLHN-NHMAK	3354	Antithrombotic peptide	DEE	[4–6]
	AGPAGPAGARGADGNVGP	3283	Antithrombotic peptide	GP	[2–3], [5–6], [17–18]
Muscle control-100% water	FDIAHTPGVAADLSHIETR	3285	Antithrombotic peptide	PG	[7–8]
	DLAHTPGVAADLSHIETR	3285	Antithrombotic peptide	PG	[6–7]
	ISKQEYDEAGPSIVHRK	3283	Antithrombotic peptide	GP	[10–11]
	RTNPGSVFDYDPAED-NIQSR	3285	Antithrombotic peptide	PG	[4–5]
	WISKQEYDEAGPSIVHR	3283	Antithrombotic peptide	GP	[11–12]

A, alanine; *C*, cysteine; *D*, aspartic acid; *E*, glutamic acid; *F*, phenylalanine; *G*, glycine; *H*, histidine; *I*, isoleucine; *K*, lysine; *L*, leucine; *M*, methionine; *N*, asparagine; *P*, proline; *Q*, glutamine; *R*, arginine; *S*, serine; *T*, threonine; *V*, valine; *W*, tryptophan; *Y*, tyrosine

GQEQSHQDEGVIVR (Lammi et al., 2018, 2019a, 2019b). For instance, two lupin peptides (LILPKHSDAD (P5) and LTFPGSAED (P7)) can act as inhibitors of HMGCoA reductase activity, both of them could increase low-density lipoprotein (LDL) receptor protein levels with enhanced LDL uptake by HepG2 cells (Zanoni et al., 2017).

In our study, the findings from sea bass side streams position them as promising sources for bioactive peptides, aligning with the growing interest in food protein-derived peptides for their health-promoting potential.

ICP-MS Analysis of Minerals

Minerals, essential for human nutrition, are categorized into two groups: macro-minerals, requiring daily intake exceeding 100 mg, including calcium (Ca), phosphorus (P), magnesium (Mg), and potassium (K), and micro-minerals, necessitating daily intake below 100 mg, such as iron (Fe), zinc (Zn), copper (Cu), among others (Cilla et al., 2019). Previous research underscored the significance of fish as a rich source of minerals, particularly Ca, P, Mg, K, Fe, and Zn (Caruso et al., 2020b; Erkan & Özden, 2007). Minerals, known for their high biocompatibility and economic value, have garnered increased attention within the food industry (Maschmeyer et al., 2020). As illustrated in Figs. 4 and 5, a substantial disparity in mineral content exists between the solid matrices and liquid extracts, with the solid matrices exhibiting higher mineral (Ca, P, Mg, K, Fe, Zn, and Cu) concentrations. Furthermore, a three-way ANOVA was conducted on the ICP-MS mineral analysis data for both solid matrices and liquid extracts, with the sea bass side stream (head, skin, viscera, and muscle), treatment (PEF or control), and solvent (100% water or 50% ethanol) as the three sources of variables, which revealed that the sea bass side stream type is the primary source of variation.

In the case of the heads, the mineral content within the solid matrices exhibited a range of values: 76.8–523.0 mg/kg for Mg, 560.0–4440.0 mg/kg for P, 255.0–1640.0 mg/

Table 2 Antioxidative activity of bioactive peptides obtained from the sea bass side streams' solid matrices extracted with 100% water or 50% ethanol (only peptides' sequences with more than 80% of confidence were selected)

Solid matrices	Sequence	ID	Name of peptide	Involved amino acid	Location
Muscle PEF-50% ethanol	AGFAGDDAPRAVFP-SIVGRPR	9443	Antioxidative peptide	AGDDAPR	[4–10]
	SSDQLGELGKGAGKGGG GGGSIR	7888	Antioxidative peptide	EL	[7–8]
		8215	Antioxidative peptide	IR	[22–23]
	MGQKDSYVGDEAQSKR-GILT	8134	Peptide derived from dried bonito	KD	[4–5]
		8496	Antioxidant peptide from ginkgo biloba	YVGD	[7–10]
	AGDDAPRAVFPSIVGRPR	9443	Antioxidative peptide	AGDDAPR	[1–7]
	DRGDSGAAGPAGPAGPAG-PRGPA	8983	Antioxidative peptide	GAA	[6–8]
Muscle PEF-100% water	GKYVSAGGSTAGGESLF-VANHAY	7866	Peptide from Okara protein	AY	[22–23]
		8114	Peptide derived from sardinelle by-product proteins (*Sardinella aurita*)	GGE	[12–14]
		10,227	Antioxidative peptide	LFV	[16–18]
	AGFAGDDAPRAVFPSIVGRP	9443	Antioxidative peptide	AGDDAPR	[4–10]
	MSADAMLKALLGSKHTVN	8217	Antioxidative peptide	LK	[7–8]
	MSADAMLKALLGSKHT-VNMDL	8217	Antioxidative peptide	LK	[7–8]
	KYVSAGGSTAGGESLFVAN-HAY	7866	Peptide from Okara protein	AY	[21–22]
		8114	Peptide derived from sardinelle by-product proteins (*Sardinella aurita*)	GGE	[11–13]
		10,227	Antioxidative peptide	LFV	[15–17]
	GVMVGMGQKDSY-VGDEAQSK	8134	Peptide derived from dried bonito	KD	[9–10]
		8496	Antioxidant peptide from ginkgo biloba	YVGD	[12–15]
	FAGDDAPRAVFPSIVGRP	9443	Antioxidative peptide	AGDDAPR	[2–8]
	VGMGQKDSYVGDEAQSKR-GIL	8134	Peptide derived from dried bonito	KD	[6–7]
		8496	Antioxidant peptide from ginkgo biloba	YVGD	[9–12]
	ANKEIEDLKIKVVDLAGVK	8217	Antioxidative peptide	LK	[8–9]
	PFLTPEQKKELSDIAQKIVAT	7888	Antioxidative peptide	EL	[10–11]
	DLEESTLQHEATAAALRK	9266	Antioxidative peptide	DLEE	[1–4]
	KGGDDLDPNYVLSSRVR	8150	Peptide derived from bovine kappa-casein (30–32)	YVL	[10–12]
	WISKQEYDEAGPSIVHR	9916	Antioxidative peptide	AGPSIVH	[10–16]
		9911	Antioxidative peptide	AGPSIVH	[10–16]
	PHAYPFLTPEQKKELSDIAQ	7866	Peptide from Okara protein	AY	[3–4]
		7888	Antioxidative peptide	EL	[14–15]
		8022	Synthetic peptide	PHA	[1–3]
	KEIEDLKLKVVDLAGVK	8217	Antioxidative peptide	LK	[6–7],[8–9]
	ISKQEYDEAGPSIVHRKC	9916	Antioxidative peptide	AGPSIVH	[9–15]
		9911	Antioxidative peptide	AGPSIVH	[9–15]
	KVAEQELVDASERVGLLH	3305		LH	[17–18]
		7888	Antioxidative peptide	EL	[6–7]

Table 2 (continued)

Solid matrices	Sequence	ID	Name of peptide	Involved amino acid	Location
	MYSQDAIMDAIAGQSQAK-GHDSG	8090	Peptide derived from sardine muscle	MY	[1–2]
		10,375	Antioxidative peptide	YSQ	[2–4]
	GGDDLDPNYVLSSRVR	8150	Peptide derived from bovine kappa-casein (30–32)	YVL	[9–11]
	VDFNVPMKDKQITNNQR	8134	Peptide derived from dried bonito	KD	[8–9]
	VFGESTAFGKADHSVS-AEKLK	8217	Antioxidative peptide	LK	[20–21]
	ANKEIEDLKIKVVDLA	8217	Antioxidative peptide	LK	[8–9]
	GKYVSAGGSTAGGESL	8114	Peptide derived from sardinelle by-product proteins (*Sardinella aurita*)	GGE	[12–14]
	N(Deamidated)LKPGELPT-CESLKDTIAR	7888	Antioxidative peptide	EL	[18–19]
		8134	Peptide derived from dried bonito	KD	[26–27]
		8216	Antioxidative peptide	LKP	[14–16]
		8217	Antioxidative peptide	LK	[14–15],[25–26]
		8218	Antioxidative peptide	KP	[15–16]
	FSVDEEFPDLSLHNNHMA	3305		LH	[12–13]
		7997	Synthetic peptide	LHN	[12–14]
	WISKQEYDEAGPSIVHRKC	9916	Antioxidative peptide	AGPSIVH	[10–16]
		9911	Antioxidative peptide	AGPSIVH	[10–16]
	AGFAGDDAPRAVFP-SIVGRPR	9443	Antioxidative peptide	AGDDAPR	[4–10]
	DNGSGLVKAGFAGDDAPRA	9443	Antioxidative peptide	AGDDAPR	[12–18]
	EQFEEEQEAKAELQR	7888	Antioxidative peptide	EL	[12–13]
		8130	Antioxidative peptide	EAK	[8–10]
	FSVDEEFPDLSLHNNHM	3305		LH	[12–13]
		7997	Synthetic peptide	LHN	[12–14]
	EIEDLKIKVVDLAGVK	8217	Antioxidative peptide	LK	[5–6]
	FSVDEEFPDLSLHNNHMAK	3305		LH	[12–13]
		7997	Synthetic peptide	LHN	[12–14]
	GIVPIVEPEILPDGDHDLK	8217	Antioxidative peptide	LK	[18–19]
		9688	Antioxidant peptide from the crucian carp (*Carassius auratus*) cooking juice	PEILPDGDHD	[8–17]
Muscle control-100% water	SADAMLKALLGSKHTVN-MDL	8217	Antioxidative peptide	LK	[6–7]
	GKYVSAGGSTAGGESLF-VANHAY	7866	Peptide from Okara protein	AY	[22–23]
		8114	Peptide derived from sardinelle by-product proteins (*Sardinella aurita*)	GGE	[12–14]
		10,227	Antioxidative peptide	LFV	[16–18]
	YSQDAIMDAIAGQSQAK-GHDSG	10,375	Antioxidative peptide	YSQ	[1–3]
	VGMGQKDSYVGDEAQSKR-GIL	8134	Peptide derived from dried bonito	KD	[6–7]
		8496	Antioxidant peptide from ginkgo biloba	YVGD	[9–12]
	YTPSFNPIALKDSALSTH	8134	Peptide derived from dried bonito	KD	[11–12]
		8217	Antioxidative peptide	LK	[10–11]
	AGFAGDDAPRAVFPSIVGRP	9443	Antioxidative peptide	AGDDAPR	[4–10]

Table 2 (continued)

Solid matrices	Sequence	ID	Name of peptide	Involved amino acid	Location
	GQKDSYVGDEAQSKRGIL	8134	Peptide derived from dried bonito	KD	[3–4]
		8496	Antioxidant peptide from ginkgo biloba	YVGD	[6–9]
	SSIFDAGAGIALNDHFVKL	8103	Peptide derived from dried bonito	VKL	[17–19]
	MSADAMLKALLGSKHT-VNMDL	8217	Antioxidative peptide	LK	[7–8]
	KYVSAGGSTAGGESLFVAN-HAY	7866	Peptide from Okara protein	AY	[21–22]
		8114	Peptide derived from sardinelle by-product proteins (*Sardinella aurita*)	GGE	[11–13]
		10,227	Antioxidative peptide	LFV	[15–17]
	FDIAHTPGVAADLSHIETR	7886	Peptide derived from egg white albumin	AH	[4–5]
	VGMGQKDSYVGDEAQSKR	8134	Peptide derived from dried bonito	KD	[6–7]
		8496	Antioxidant peptide from ginkgo biloba	YVGD	[9–12]
	VRMSADAMLKALLG-SKHTV	8217	Antioxidative peptide	LK	[9–10]
	GKYVSAGGSTAGGESLFVA N(Deamidated)HAY	7866	Peptide from Okara protein	AY	[34–35]
		8114	Peptide derived from sardinelle by-product proteins (*Sardinella aurita*)	GGE	[12–14]
		10,227	Antioxidative peptide	LFV	[16–18]
	NKEIEDLKIKVVDLA	8217	Antioxidative peptide	LK	[7–8]
	IEDLKIKVVDLAGVK-KPALK	8217	Antioxidative peptide	LK	[4–5],[19–20]
		8218	Antioxidative peptide	KP	[16–17]
	KANKEIEDLKIKVVD-LAGVK	8217	Antioxidative peptide	LK	[9–10]
	MSADAMLKALLGSKHT-VNM	8217	Antioxidative peptide	LK	[7–8]
	N(Deamidated)ASVI-PEGQFIDNKKASEKLLG	8217	Antioxidative peptide	LK	[6–7]
	SADAMLKALLGSKHTVN	8217	Antioxidative peptide	LK	[6–7]
	MYSQDAIMDAIAGQSQAK-GHDSG	8090	Peptide derived from sardine muscle	MY	[1–2]
		10,375	Antioxidative peptide	YSQ	[2–4]
	NKEIEDLKIKVVDLAGVK	8217	Antioxidative peptide	LK	[7–8]
	KISVPKDVMMEELNLPSNR	7888	Antioxidative peptide	EL	[12–13]
		8134	Peptide derived from dried bonito	KD	[6–7]
		9086	Antioxidative peptide	MM	[9–10]
	IEDLKIKVVDLAGVKKPAL	8217	Antioxidative peptide	LK	[4–5]
		8218	Antioxidative peptide	KP	[16–17]
	TPSFNPIALKDSALSTHKPIE	8134	Peptide derived from dried bonito	KD	[10–11]
		8217	Antioxidative peptide	LK	[9–10]
		8218	Antioxidative peptide	KP	[18–19]
	HQGVMVGMGQKDSY-VGDEAQSK	8134	Peptide derived from dried bonito	KD	[11–12]
		8496	Antioxidant peptide from ginkgo biloba	YVGD	[14–17]

Table 2 (continued)

Solid matrices	Sequence	ID	Name of peptide	Involved amino acid	Location
	KYVSAGGSTAGGESLFVAN(Deamidated)HAY	7866	Peptide from Okara protein	AY	[33–34]
		8114	Peptide derived from sardinelle by-product proteins (*Sardinella aurita*)	GGE	[11–13]
		10,227	Antioxidative peptide	LFV	[15–17]
	SSDQLGELGKGAGKGGGGGGSIR	7888	Antioxidative peptide	EL	[7–8]
		8215	Antioxidative peptide	IR	[22–23]
	MSADAMLKALLGSKHTVN	8217	Antioxidative peptide	LK	[7–8]
	TPSFNPIALKDSALSTH	8134	Peptide derived from dried bonito	KD	[10–11]
		8217	Antioxidative peptide	LK	[9–10]
	TPSFNPIALKDSALS	8134	Peptide derived from dried bonito	KD	[10–11]
		8217	Antioxidative peptide	LK	[9–10]
	KGTLKAVDHVNKDIAPKLIA	8134	Peptide derived from dried bonito	KD	[12–13]
		8217	Antioxidative peptide	LK	[4–5]
	GQKDSYVGDEAQSKRGILT	8134	Peptide derived from dried bonito	KD	[3–4]
		8496	Antioxidant peptide from ginkgo biloba	YVGD	[6–9]
	DNGSGLVKAGFAGDDAPR	9443	Antioxidative peptide	AGDDAPR	[12–18]
	AGFAGDDAPRAVFPSIVGRPR	9443	Antioxidative peptide	AGDDAPR	[4–10]
	GQKDSYVGDEAQSKRGILTL	8134	Peptide derived from dried bonito	KD	[3–4]
		8496	Antioxidant peptide from ginkgo biloba	YVGD	[6–9]
	SADAMLKALLGSKHTVNM	8217	Antioxidative peptide	LK	[6–7]
	Q(Deamidated)LGELGKGAGKGGGGGGSIR	7888	Antioxidative peptide	EL	[16–17]
		8215	Antioxidative peptide	IR	[31–32]
	MGQKDSYVGDEAQSKRGIL	8134	Peptide derived from dried bonito	KD	[4–5]
		8496	Antioxidant peptide from ginkgo biloba	YVGD	[7–10]
	KANKEIEDLKIKVVDLA	8217	Antioxidative peptide	LK	[9–10]
	SADAMLKALLGSKHTVNMDLR	8217	Antioxidative peptide	LK	[6–7]
	ANKEIEDLKIKVVDLA	8217	Antioxidative peptide	LK	[8–9]
	SSGDLLRAEVASGSE	8484	Antioxidant peptide from as1-CN (98–100)	LLR	[5–7]
	DLAHTPGVAADLSHIETR	7886	Peptide derived from egg white albumin	AH	[3–4]
	SAAGGESLFVANHAY	7866	Peptide from Okara protein	AY	[14–15]
		8114	Peptide derived from sardinelle by-product proteins (*Sardinella aurita*)	GGE	[4–6]
		10,227	Antioxidative peptide	LFV	[8–10]
	SGASAAGGESLFVANHA	8114	Peptide derived from sardinelle by-product proteins (*Sardinella aurita*)	GGE	[7–9]
		10,227	Antioxidative peptide	LFV	[11–13]
	PHAYPFLTPEQKKELSDIA	7866	Peptide from Okara protein	AY	[3–4]
		7888	Antioxidative peptide	EL	[14–15]

Table 2 (continued)

Solid matrices	Sequence	ID	Name of peptide	Involved amino acid	Location
		8022	Synthetic peptide	PHA	[1–3]
	MHGIVPIVEPEILPDGDH-DLK	8217	Antioxidative peptide	LK	[20–21]
		9688	Antioxidant peptide from the crucian carp (*Carassius auratus*) cooking juice	PEILPDGDHD	[10–19]
	VGKANKEIEDLKIKVVDL	8217	Antioxidative peptide	LK	[11–12]
	GIVPIVEPEILPDGDHDLKR	8217	Antioxidative peptide	LK	[18–19]
		9688	Antioxidant peptide from the crucian carp (*Carassius auratus*) cooking juice	PEILPDGDHD	[8–17]
	VEYTPSFNPIALKDSALSTH	8134	Peptide derived from dried bonito	KD	[13–14]
		8217	Antioxidative peptide	LK	[12–13]
	VRMSADAMLKALLG-SKHTVN	8217	Antioxidative peptide	LK	[9–10]
	KFAGKDYRHPKIN	8134	Peptide derived from dried bonito	KD	[5–6]
	KEIEDLKIKVVDLA	8217	Antioxidative peptide	LK	[6–7]
	ISKQEYDEAGPSIVHRK	9916	Antioxidative peptide	AGPSIVH	[9–15]
		9911	Antioxidative peptide	AGPSIVH	[9–15]
	GKYVSAGGSTAGGESL	8114	Peptide derived from sardinelle by-product proteins (*Sardinella aurita*)	GGE	[12–14]
	GIVPIVEPEILPDGDHDLK	8217	Antioxidative peptide	LK	[18–19]
		9688	Antioxidant peptide from the crucian carp (*Carassius auratus*) cooking juice	PEILPDGDHD	[8–17]
	IVPIVEPEILPDGDHDLK	8217	Antioxidative peptide	LK	[17–18]
		9688	Antioxidant peptide from the crucian carp (*Carassius auratus*) cooking juice	PEILPDGDHD	[7–16]
	WISKQEYDEAGPSIVHR	9916	Antioxidative peptide	AGPSIVH	[10–16]
		9911	Antioxidative peptide	AGPSIVH	[10–16]
	AAVPSGASTGVHEALELR	7888	Antioxidative peptide	EL	[16–17]
	GVMVGMGQKDSY-VGDEAQSK	8134	Peptide derived from dried bonito	KD	[9–10]
		8496	Antioxidant peptide from ginkgo biloba	YVGD	[12–15]
	IFDAGAGIALNDHFVKL	8103	Peptide derived from dried bonito	VKL	[15–17]
	VRMSADAMLKALLGSKHT	8217	Antioxidative peptide	LK	[9–10]
	VRMSADAMLKALLGS	8217	Antioxidative peptide	LK	[9–10]

A, alanine; *C*, cysteine; *D*, aspartic acid; *E*, glutamic acid; *F*, phenylalanine; *G*, glycine; *H*, histidine; *I*, isoleucine; *K*, lysine; *L*, leucine; *M*, methionine; *N*, asparagine; *P*, proline; *Q*, glutamine; *R*, arginine; *S*, serine; *T*, threonine; *V*, valine; *W*, tryptophan; *Y*, tyrosine

kg for K, 384.0–2060.0 mg/kg for Ca, 229.9–77.6 mg/kg for Fe, and 18.66–27.9 mg/kg for Zn (Fig. 5). According to the results of the three-way ANOVA, the source of variation primarily responsible for the total variation of Mg was the solvent (19.04%); for P, the interaction sea bass side steam × solvent (24.5%); for K, the solvent (9.8%); for Ca, the interaction sea bass side stream × solvent (26.8%); for Fe, the interaction sea bass side stream × solvent (13.51%); and for Zn, the interaction sea bass side stream × solvent (9.7%). Notably, the choice of solvent had a pronounced impact on mineral content in the solid matrices, with the use of 50% ethanol resulting in a significant increase compared to the use of 100% water. Conversely, the treatment method did not significantly influence mineral content. In

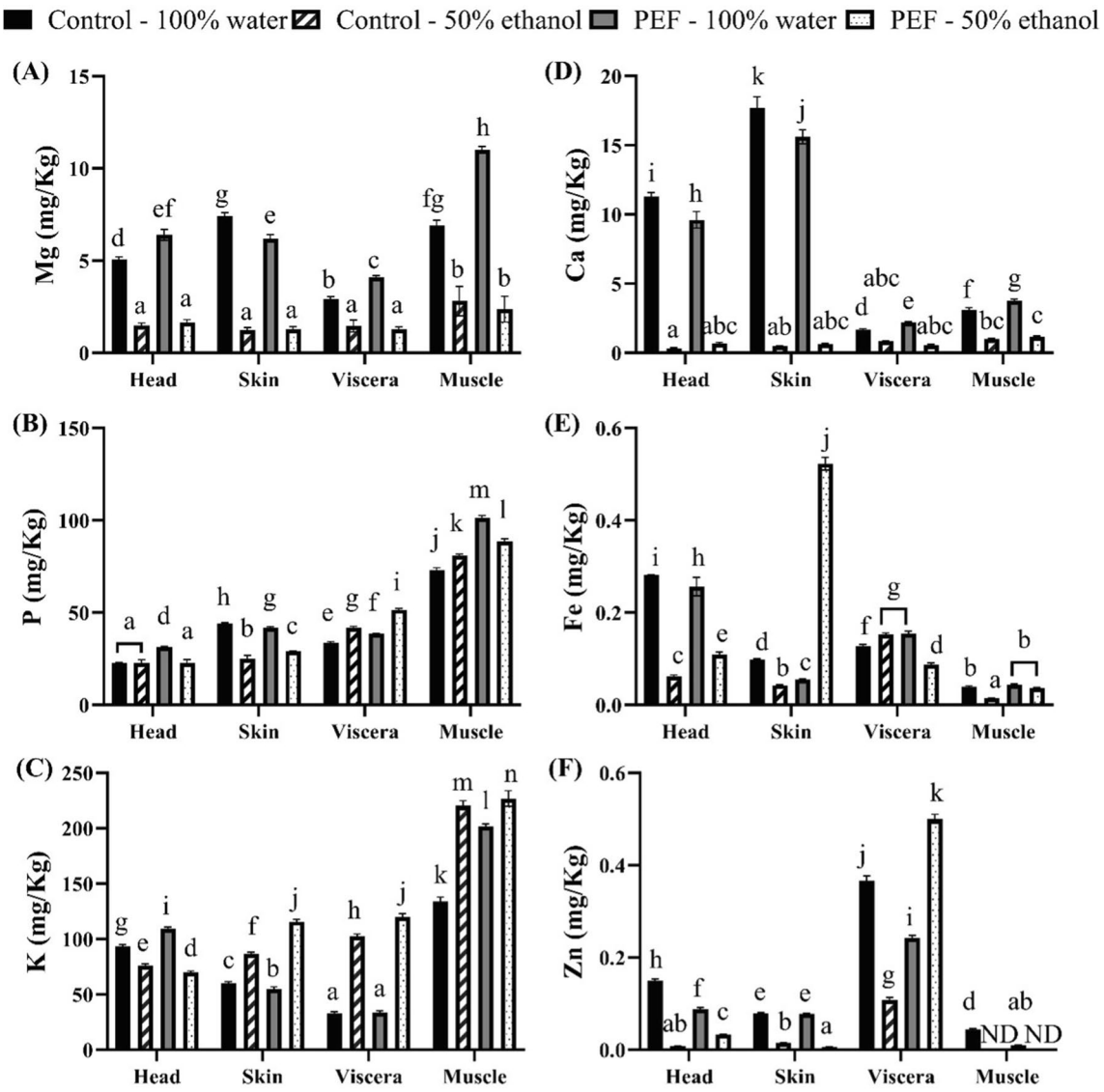

Fig. 4 Mineral contents in liquid extracts obtained from sea bass side streams extracted with 100% water or 50% ethanol. Significant differences between different data are represented by different letters, starting with the smallest value marked with a

terms of the liquid extracts, the mineral content in the head ranged from 1.5 to 6.4 mg/kg for Mg, 22.7 to 31.4 mg/kg for P, 75.7 to 109.0 mg/kg for K, 0.3 to 11.3 mg/kg for Ca, 0.062 to 0.282 mg/kg for Fe, and 0.007 to 0.150 mg/kg for Zn (Fig. 4).

In the case of the skin, the mineral content within the solid matrices displayed a range of values: 74.6–523.0 mg/kg for Mg, 450.0–20,500.0 mg/kg for P, 88.0–1300.0 mg/kg for K, 561.0–37,100.0 mg/kg for Ca, 6.6–11.5 mg/kg for Fe, and 28.7–144.0 mg/kg for Zn (Fig. 5). Notably, the choice of solvent significantly influenced the mineral content remaining in the solid matrices or passing in the liquid extracts. Particularly, the lowest levels of mineral recovery or higher values remaining in solid matrices (Mg, P, Ca, and Zn) were observed when 50% ethanol was used as the extraction solvent. Additionally, the PEF treatment emerged as a significant source of variation, leading to a notable decrease in mineral content for the skin solid matrices, especially when compared with the control extraction performed with the same solvent. Concerning the liquid extracts, the mineral content in the skin ranged from 1.2 to 7.4 mg/kg for Mg, 25.0 to 44.1 mg/kg for P, 54.8 to 115.8 mg/kg for K, 0.5 to 17.7 mg/kg for Ca, 0.043 to 0.523 mg/kg for Fe, and 0.005 to 0.079 mg/kg for Zn (Fig. 4). It is worth noting that lower values in the liquid extracts (Mg, P, Ca, and Zn) were obtained when extracted with 100% water (Fig. 4). The enhancement in the mineral recovery in the liquid extracts following PEF treatment

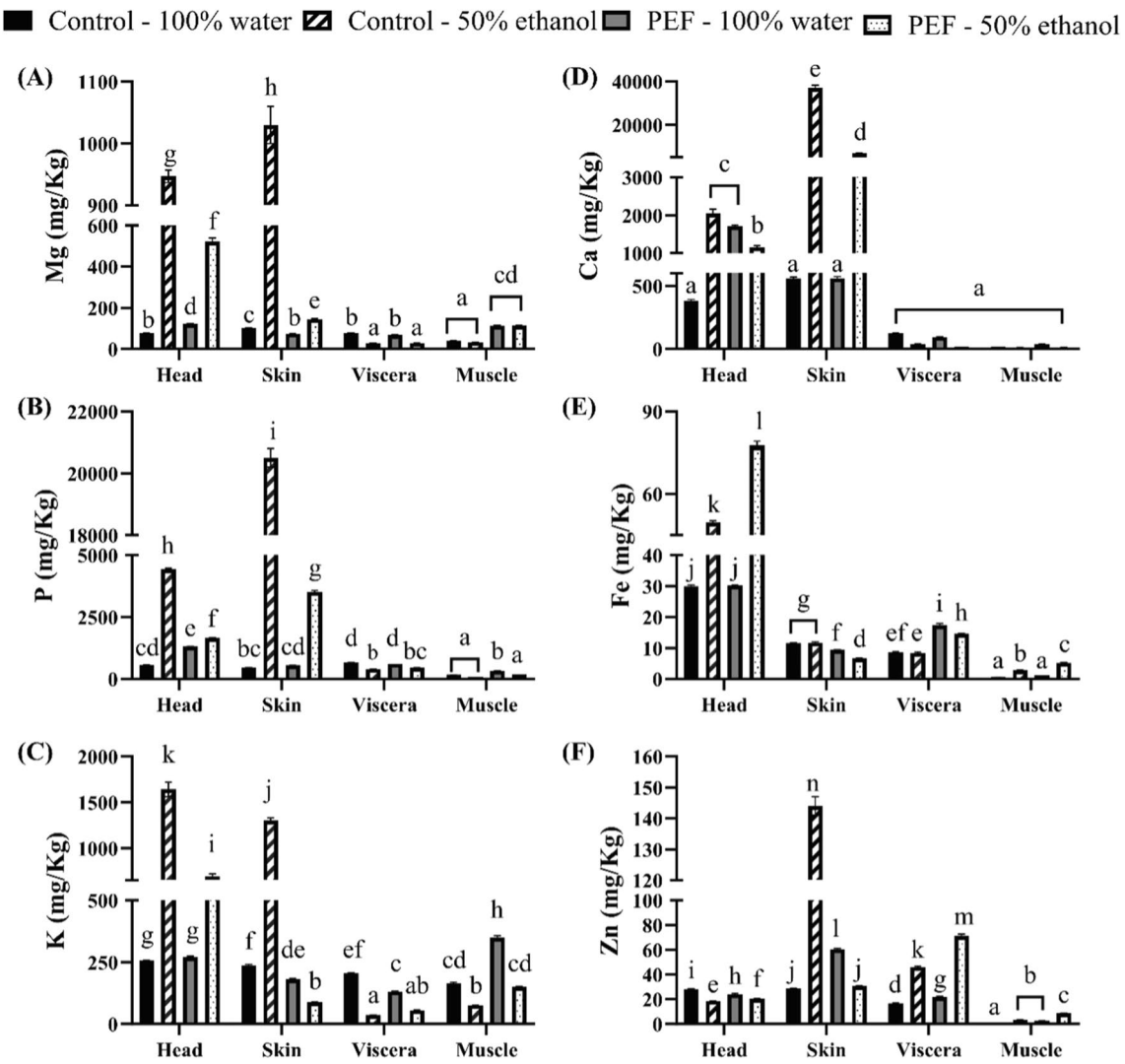

Fig. 5 Mineral contents in solid matrices obtained from sea bass side streams extracted with 100% water or 50% ethanol. Significant differences between different data are represented by different letters, starting with the smallest value marked with a

was only observed for the K and Zn using 50% ethanol as the solvent (Fig. 4).

As for the viscera, the remaining mineral content within the solid matrices ranged from 28.4 to 77.7 mg/kg for Mg, 399.0 to 650.0 mg/kg for P, 35.8 to 206.0 mg/kg for K, 18.4 to 124.5 mg/kg for Ca, 8.4 to 17.4 mg/kg for Fe, and 16.7 to 71.3 mg/kg for Zn (Fig. 5). Notably, a decrease in mineral content in the solid matrixes was observed for Mg, P, K, Ca, and Fe when 50% ethanol was used as the extraction solvent. Meanwhile, the treatment method did not significantly impact the mineral content in the solid matrices (Mg, P, K, and Ca) when comparing the control and PEF treatment. In terms of the liquid extracts, the mineral content in the viscera ranged from 1.5 to 4.1 mg/kg for Mg, 33.7 to 51.5 mg/kg for P, 33.1 to 119.8 mg/kg for K, 0.8 to 2.2 mg/kg for Ca, 0.088 to 0.155 mg/kg for Fe, and 0.107 to 0.500 mg/kg for Zn (Fig. 4). Notably, higher values in the liquid extracts (Mg and Ca) were obtained when 100% water was used as the solvent. Furthermore, PEF treatment resulted in an increase in Mg, P, Ca, and Fe content in the liquid extracts using 100% water and P, K, and Zn using 50% ethanol as the solvent.

As for the muscle, the mineral content within the solid matrices ranged from 33.6 to 114.0 mg/kg for Mg, 72.9 to 328.0 mg/kg for P, 76.1 to 165.0 mg/kg for K, 10.8 to 38.7 mg/kg for Ca, 0.5 to 5.2 mg/kg for Fe, and 0.6 to 8.5 mg/kg for Zn (Fig. 5). In terms of the liquid extracts, the mineral content in muscle ranged from 2.4 to 11.0 mg/kg for Mg, 72.9 to 101.2 mg/kg for P, 133.8 to 226.8 mg/kg for K, 1.0 to 3.8 mg/kg for Ca, 0.015 to 0.44 mg/kg for Fe, and 0.010 to 0.044 mg/kg for Zn (Fig. 4). Mineral recovery in the liquid extracts of sea bass muscle was higher using 100% water as the solvent for Mg, Ca, Fe, and Zn, with or without

additional PEF treatment. Mineral recovery in the muscle extracts was enhanced by the use of 50% ethanol only in the case of K, both without and even more after PEF treatment.

In this study, it is evident that the sea bass side stream type was the primary source of variation in the total mineral content, as determined by ICP-MS analysis. Subsequently, the choice of solvent emerges as a crucial factor influencing mineral composition within each sea bass side stream. Notably, the mineral content remaining in solid matrices was higher when 50% ethanol was used as the extraction solvent, and accordingly, the highest mineral content recovered in liquid extracts was achieved when 100% water was employed. The application of PEF pre-treatment did, but not in all cases, result in a significant shift in the mineral within the solid matrices and liquid extracts. Accordingly, previous research indicated that PEF processing can lead to the recovery of higher mineral quantities, including minerals such as Ca, K, P, Fe, Na, and Mg, attributed to the substantial release of minerals resulting from membrane permeabilization induced by PEF treatment (Wang et al., 2021a). Moreover, prior research demonstrated that there are no adverse alterations in mineral release observed in control meat samples with PEF treatment during in vitro gastrointestinal digestion (Bhat et al., 2019). Meat is recognized as a substantial source of essential minerals, and any potential changes in mineral content could have adverse implications for commercial purposes. Consequently, the application of PEF technology has been explored as a means to enhance the concentration of various minerals in meat products (Gómez et al., 2019). Nonetheless, it is important to note that in this study, the treatment (PEF) accounted for a modest 2.31% of the total variation in the mineral content of liquid extracts, specifically in the case of P. Furthermore, when combined with other sources of variation, such as the sea bass side stream and/or solvent, the treatment can influence the mineral content in both solid matrices (P, K, Fe, and Zn) and liquid extracts (Fe and Zn). Considering these findings, it can be surmised that solid matrices extracted with 50% ethanol can indeed serve as valuable sources of Mg, P, K, Ca, Fe, and Zn for consumption.

ICP-MS Analysis of Heavy Metal

In general, heavy metals are common pollutants in the environment and are sometimes harmful to human health (Kazemi et al., 2022b; Rathi et al., 2021). Even low intake levels of non-essential heavy metals, such as arsenic (As), cobalt (Cd), mercury (Hg), and lead (Pb), can impair human neurological, respiratory, and cardiovascular function (Varol et al., 2021). Nevertheless, humans need essential metals for daily physiological metabolism, such as Zn and chromium (Cr). The heavy metal content of As, Cd, Hg, and Pb in the liquid extracts and solid matrices investigated in this work is shown in Fig. 6.

In terms of As, the highest content in liquid extracts and solid matrices was 48.5 µg/L and 1159.0 µg/kg, respectively. For the same matrices in liquid extracts, the higher Cd content was obtained using 100% water as the solvent, increasing further following PEF treatment.

In terms of Cd, Hg, and Pb, the respective heavy metal levels in liquid extracts and the respective values remaining in the solid matrices are presented in Fig. 6. The head, skin, viscera, and muscle samples contained lower amounts of Cd, Hg, and Pb as compared to the As values (Fig. 6). Higher heavy metal recovery was achieved in skin, viscera, and muscle liquid extracts using 50% ethanol as the solvent without PEF treatment, and the respective concentrations of Cd, Hg, and Pb in solid matrices were below 117.0 µg/kg. On the other hand, higher heavy metal recovery levels were found following PEF treatment when 100% water was used as the solvent. Consequently, the analyzed values of heavy metals remaining in solid matrices (Cd, Hg, and Pb) (Fig. 6) were lower than 100.0 µg/kg when 100% water was used as the extraction solvent.

The existing regulatory limits for As, Hg, Cd, and Pb in wet fish food products are 13.5 mg/kg, 0.5 mg/kg, 0.05 mg/kg, and 0.3 mg/kg, respectively (Kandyliari et al., 2021; Renieri et al., 2019), which are above the levels found in the samples of our study. As a result, all solid matrices and liquid extracts obtained from sea bass side streams (head, skin, viscera, and muscle) using either 100% water or 50% ethanol as the solvent can be considered safe ingredients to be used by the food industry enabling circular economy performance of the sector.

Side Stream Fraction Total Antioxidant Capacity (TAC)

To evaluate the quality of the obtained liquid extracts from sea bass, the TEAC values were determined and the results are shown in Fig. 7 (A). According to the three-way ANOVA, the three main factors that account for the total variation of the variance are the sea bass side stream with 71.92%, the interaction sea bass side stream × solvent which accounted for 20.24% of the total variation, and the solvent which accounted for 2.12%.

As can be seen in Fig. 7 (A) and (B), the viscera liquid extracts following PEF pre-treatment present the highest TEAC and ORAC as compared to the other side stream fractions tested (head, skin, and muscle). When 50% ethanol was used as the extraction solvent, viscera-PEF reached the highest TEAC value (503.8 µM) analyzed in this study. The interaction of sea bass side stream and solvent represented the factor responsible for the second highest variation source

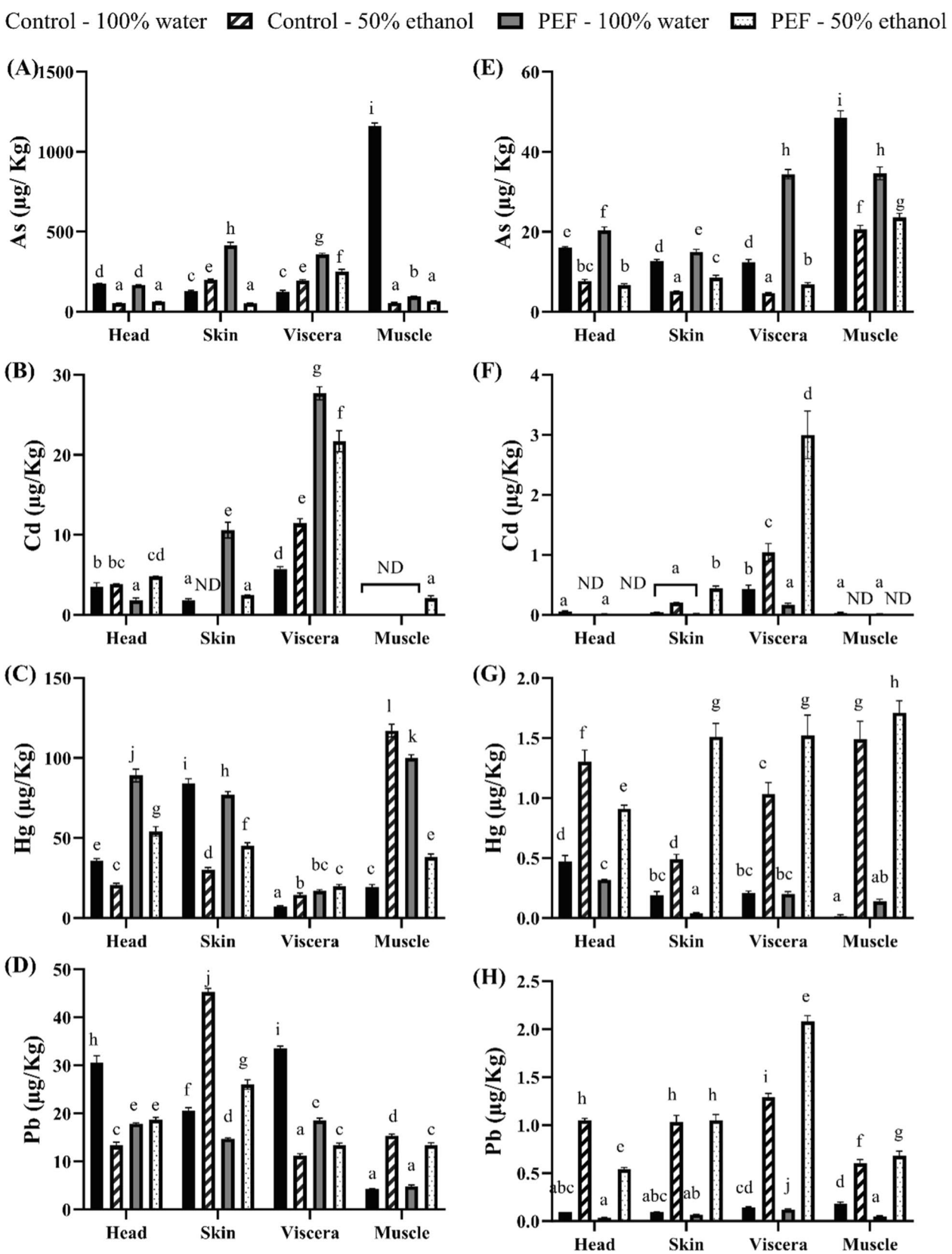

Fig. 6 Heavy metal in solid matrices and liquid extracts obtained from sea bass side streams extracted with 100% water or 50% ethanol. Significant differences between different data are represented by different letters, starting with the smallest value marked with a. Solid matrices include A, B, C, and D; liquid extracts include E, F, G, and H

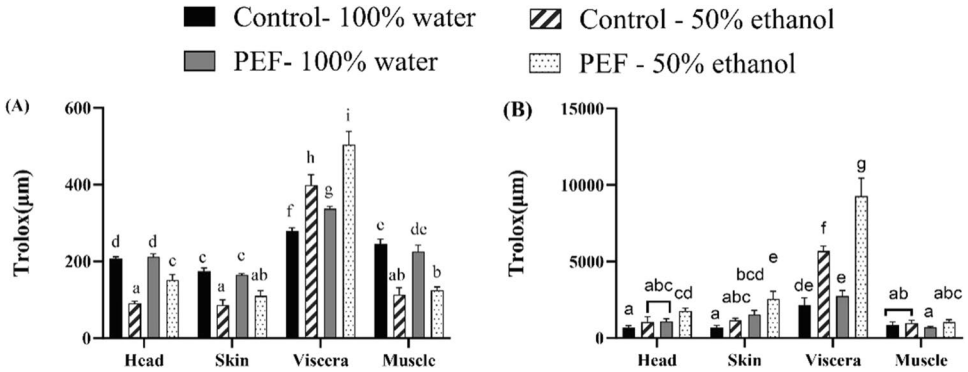

Fig. 7 Trolox equivalent antioxidant capacity (TEAC) and oxygen radical absorbance capacity (ORAC) in liquid extracts obtained from sea bass side streams extracted with 100% water or 50% ethanol. (A) TEAC; (B) ORAC. Significant differences between different data are represented by different letters, starting with the smallest value marked with a

(20.24%) in this part of the study, with 100% water used as the solvent yielding higher TEAC values in the extracts as compared to the 50% ethanol treatments in most of the side stream types used (muscle, skin, head), whereas the opposite was observed processing viscera samples. PEF treatment led to increased TEAC only in the viscera side stream samples.

TEAC is a common method used in evaluating antioxidant capacity of solutions and the principle of the method is that the test material compounds react directly with ABTS +, or intercept oxidation and prevent the production of ABTS + (Schaich et al., 2015). The application of PEF technology significantly increased the ability of viscera extracts to resist the production of ABTS + ($p < 0.05$) by 20.40% compared to the control (Fig. 7 (A)). Contrary to our results, a recent study (Wang et al., 2021a) concluded that PEF significantly degraded ($p < 0.05$) the ABTS + scavenging ability of viscera extract in sole and not impacted the viscera liquid extracts in rainbow trout ($p > 0.05$). This may be due to differences in the fish species and matrices. The present study provides evidence that the fish side streams are a source of highly active peptides with superior antioxidant potential (Franco et al., 2020; Martí-Quijal et al., 2023). The sea bass side streams (heads, skins, viscera, and muscle) whether or not subjected to PEF treatment demonstrated a considerable antioxidant capacity. Overall, it is found that the PEF treatment can improve the TEAC of the viscera liquid extracts, with even stronger effects using 50% ethanol used as the extraction solvent as compared to only water.

A comparative study of the analytical methods for the evaluation of the antioxidant activity of liquid food employing spectrophotometry, fluorometry, and chemiluminescence shows that there is a correlation between the results of the antioxidant capacity evaluation assays TEAC and ORAC (Barba et al., 2013; Zhou et al., 2021).

In our present study, the sea bass side stream type, sea bass side stream type × solvent, and solvent explained 54.54%, 19.45%, and 13.53% of the total variation in the ORAC assay results. As shown in Fig. 7 (B), the viscera displayed an extraordinary ORAC antioxidant capacity highest following PEF treatment using 50% ethanol as the solvent (9278.84 μM). In total, using 50% ethanol as the solvent significantly enhanced ($p > 0.05$) the ORAC of fish side stream extracts as compared to 100% water. Previous researchers have also investigated TEAC for the sea bass side streams obtained by PEF optimum condition with the high value (~ 3000 μM TE/L) (Martí-Quijal et al., 2023).

Furthermore, PEF treatment accounted for 4.42% of the total variation in the ORAC assay results and significantly increased ORAC of fish side stream extracts, not in all cases statistically significantly. For instance, PEF treatment did not promote the antioxidant compound extraction from sea bass muscle but led to a tendency or significant increase in the ORAC of the tested side stream materials. Using PEF and water or ethanol as extraction solvents can promote environmentally sustainable processing of fish side stream biomass for application in the food industry. The PEF and accelerated solvent extraction (ASE) were applied as the extraction technologies to verify the ORAC and ABTS in previous research studies, revealing that the antioxidant capacity of rainbow trout and sole skin and head liquid extracts was significantly raised following both PEF and ASE processing (Wang et al., 2021a). Similarly, the PEF process for obtaining antioxidant extracts from sea bream and sea bass side streams (heads, bones, and gills) was confirmed by the ORAC, DPPH, and FRAP tests, but in the case of ORAC, using methanol as the solvent yielded higher values as compared to water (Franco et al., 2020).

In our study, the matrices were the main factor affecting the obtained ORAC values, and 50% ethanol applied as the extraction solvent performed better than 100% water. Especially, the PEF treatment demonstrated a significant enhancement for viscera matrices and a more moderate improvement in the remaining side stream materials.

Conclusions

The valorization of sea bass side streams by recovering high-added-value peptide compounds holds significant promise in the fish industry. Fish side stream biomass quality variability

can be counteracted by systematic fractionation of the biomass in more homogenous materials, such as heads, skin, viscera, and muscle. This study marked the first attempt to identify and quantify various bioactive compounds in different solid matrices and liquid extracts, aided by PEF pre-treatment and employing different extraction solvents. PEF technology was as pre-treatment processing, with 100% water and 50% ethanol as extraction solvents enhanced in many cases the recovery of high-added-value compounds from sea bass side streams, including bioactive peptides and potentially other antioxidant compounds.

Both 100% water or 50% ethanol solvents and PEF treatment effectively boost protein recovery in liquid extracts obtained from sea bass side streams, while substantial protein amounts remained in the solid matrices. Recovery of both essential (Ca, Mg, P, K, Fe, and Zn) minerals and heavy metals (Cd, Hg, and Pb) in the liquid extracts was enhanced when 50% ethanol was used as an extraction solvent. Recovery of As in the liquid extract was higher with 100% water.

Using TEAC and ORAC assays, we demonstrated that PEF treatment significantly enhances the functionality of fish side stream extracts using 100% water as the solvent, except for viscera extracts in which antioxidant capacity (ORAC) was higher using 50% ethanol as the solvent.

Our study can contribute in the development of a food ingredient database of both liquid and solid remains of fish side stream processing. Of course, this study only focuses on the potent component, bioactivity peptides, minerals, and heavy metals. More studies are needed to clarify the other compounds (like fat, collagen, and vitamin E) can be recovered and bioaccessibility can be determined in further research.

Author Contribution Houda Berrada, Francisco J. Barba and Juan M. Castagnini: Conceptualization; Yixuan Liu, Min Wang, Jianjun Zhou: Formal analysis; Yixuan Liu, Francisco J. Barba, Juan M. Castagnini: Funding acquisition; Yixuan Liu: Investigation; Yixuan Liu, Min Wang, Jianjun Zhou: Methodology; Katerina Kousoulaki, Francisco J. Barba: Project administration; Houda Berrada, Katerina Kousoulaki, Francisco J. Barba: Resources; Houda Berrada, Francisco J. Barba, Juan M. Castagnini: Supervision; Yixuan Liu: Writing – original draft; Yixuan Liu, Houda Berrada, Min Wang, Jianjun Zhou, Katerina Kousoulaki, Francisco J. Barba, Juan M. Castagnini: Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. This research has been funded by BBI-JU through the H2020 project AQUABIOPRO-FIT "Aquaculture and agriculture biomass side stream proteins and bioactives for feed, fitness, and health promoting nutritional supplements" (Grant number 790956). The authors also received financial support from Generalitat Valenciana (IDIFEDER/2018/046—Procesos innovadores de extracción y conservación: pulsos eléctricos y fluidos supercríticos) through the European Union ERDF funds (European Regional Development Fund). Yixuan Liu was supported by a Ph.D. fellowship from the China Scholarship Council (CSC) (No. 202208420013). Moreover, Juan Manuel Castagnini is a beneficiary of the grant (ZA21-028) for the requalification of the Spanish university system from the Ministry of Universities of the Government of Spain, modality "Maria Zambrano" financed by the European Union, NextGeneration EU.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of Interest The authors declare no competing interests.

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