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RESEARCH

Enzymatic Hydrolysis Enhances Antioxidant Bioactive Sequences and Mineral Recovery from SC-CO_2 Defatted Salmon Side Streams

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Abstract

Efficient extraction methods are needed to maximize the recovery of high-added-value components for sustainable utilization. This study aimed to compare enzymatic hydrolysis (EH) with conventional extraction (CE) in recovering proteins, minerals, and antioxidant compounds from defatted salmon side streams after supercritical fluid extraction (SFE: 25.0 MPa, 40 °C, 75 min, 10.0 mL/min). Protein content, bioactive peptides, mineral composition, bioaccessibility, and total antioxidant capacity (TAC) were analyzed in solid matrices and liquid extracts from head, backbone, and viscera. EH-derived liquid extracts had significantly higher protein content than CE. Antioxidant activity was highest in solid matrices (head and backbone). Bioactive peptides with anti-inflammatory, immunomodulatory, and hypolipidemic properties were identified. The mineral content varied across the side streams, and the viscera showed the highest Fe, Cu, Zn, and Se levels. Mineral bioaccessibility ranged widely (0.1–100.0%), suggesting variability in bioavailability. EH outperforms CE in recovering essential nutrients and bioactive compounds, demonstrating its potential for valorizing salmon side streams. Further research should optimize EH conditions to enhance mineral bioavailability and explore industrial-scale applications for food, nutraceutical, or feed ingredients.

Keywords Enzymatic hydrolysis (EH) · Salmon side streams · Defatted · Bioactive compound · Mineral

Introduction

Protein-rich fish processing side streams, or rest raw materials (RRM), comprise about 60% of total fish biomass, yet a significant portion is used for low-added-value products like fish meal and silage, despite the bioactive potential of protein hydrolysates for functional foods and nutraceuticals development (Kaushik et al., 2024). Fish side streams hold immense potential for recovering high-added-value compounds which can be used in human health applications or the food industry (Ishak & Sarbon, 2018). Fish processing side streams, such as trimmings, viscera, and frames of commercial fish species, are rich in proteins and bioactive peptides (BPs), offering economical sources for food and nutraceutical industries (Abachi et al., 2023; Kristinsson & Rasco, 2000; Nikoo et al., 2023; Šimat, 2021).

BPs generated from food proteins have great potential as food additives in newly formulated products to enhance their functional and nutritional properties and also pointed out potential applications in food and pharmaceuticals (López-Pedrouso et al., 2023; Zaky et al., 2022). Fish protein hydrolysate (FPH) is known to be an excellent source of essential nutrients and antioxidant peptides (Vázquez et al., 2020). Fish viscera (12–18% of fish weight) contain high-value compounds, with hydrolysates showing antioxidant, ACE-inhibitory, and antibacterial activities (Huang et al., 2024). Various advanced techniques, such as

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pulsed electric fields (PEF), pressurized liquid extraction (PLE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), enzymatic hydrolysis (EH), accelerated solvent extraction (ASE), and combined PEF + SFE, improve recovery of these compounds (Martí-Quijal et al., 2023; Wang et al., 2021).

EH is widely recognized for its mild, eco-friendly nature and precise control over peptide formation, which enhances the bioactivity potential of the resulting hydrolysates (Yathisha et al., 2023). Unlike alkali, acid, and thermal methods, EH closely mimics the natural digestive process by breaking down fish proteins into hydrolysates using endogenous and/or commercial enzymes, such as alcalase, papain, and bromelain (Kaushik et al., 2024; Yathisha et al., 2023).

The optimization of hydrolysis conditions for producing protein hydrolysates from trout (*Oncorhynchus mykiss*), anchovy (*Engraulis encrasicolus*), and whiting (*Merlangius merlangus*) wastes using response surface methodology revealed that trout achieved the highest hydrolysis degree (74.3%), while whiting had the lowest (50.9%) Flavourzyme® (50.9%) (Korkmaz & Tokur, 2022). Further characterization of purified peptides, including molecular weight, amino acid sequence, and composition, demonstrated that peptides with low molecular weights and short amino acid sequences exhibit greater bioactive potential (Ishak & Sarbon, 2018).

Additionally, lipid-rich hydrolysates may influence the functional and bioactive properties of the peptides (Chemat et al., 2020; Yathisha et al., 2023). Therefore, defatted fish side streams represent promising samples for EH, as the extraction process can enhance the recovery of nutrients and bioactive compounds, not only proteins and their derivatives. Previous studies highlight supercritical fluid extraction (SFE), especially using supercritical carbon dioxide (SC-CO_2), as a sustainable and efficient method for recovering high-value bioactive compounds from food by-products (Zhang & Wu, 2025). SFE was employed to defat fish side streams due to its eco-sustainable nature, relying solely on carbon dioxide (CO_2) and ethanol as solvents (Liu et al., 2025b). This green technology has shown promise; for instance, hydrolysates derived from defatted large yellow croaker roe exhibited potent antioxidant activity (Zhang et al., 2023). Similarly, other research has found that ACE-I inhibitory activity is enhanced in lipid-free ribbon fish (*Lepturacanthus savala*) protein hydrolysates, suggesting potential applications in developing functional foods (Yathisha et al., 2023).

In this study, the recovery of proteins from lipid-rich matrices, such as fish processing side streams, requires efficient defatting as a critical preliminary step. This work pioneers a circular bioeconomy strategy by applying SFE as a pretreatment to delipidate salmon side streams, including heads, backbones, and viscera. The resulting "SFE solid cakes" were then subjected to EH, generating two main product streams: (1) solid matrices rich in proteins, BPs, and minerals, and (2) liquid extracts, which were analyzed for antioxidant capacity, mineral content, and BPs. Beyond protein recovery, we investigated the bioaccessibility of minerals in defatted matrices, assessing their potential as functional food ingredients. By merging SFE and EH, this work not only enhances valorization but also sets a blueprint for zero-waste biorefining of marine side streams.

Materials and Methods

Samples

Freeze-dried Atlantic salmon (*Salmo salar*) was provided by the Department of Nutrition and Feed Technology at Nofima (Bergen, Norway). The salmon was processed locally to separate the head, backbones, and viscera, which were subsequently freeze-dried, ground into powder using a Pulverisette 11 (Fritsch, Germany), and stored at − 20 °C for future analyses. The entire lipid-free treatment and enzymatic hydrolysis process for the salmon side streams (head, backbone, and viscera) is outlined in Fig. 1.

Chemicals and Reagents

The Protamex® (protease from *Bacillus* sp.) was purchased from Sigma Chemicals Co. (St Louis, MO, USA). ABTS, APPH, and sodium nitrate (≥ 99% purity) were purchased from Sigma-Aldrich. Deionized water was obtained using the Milli-Q SP Reagent Water System (Millipore Corporation, Bedford, MA, USA). The simulated salivary fluid (SSF, pH = 7), simulated gastric fluid (SGF, pH = 3), and simulated intestinal fluid (SIF, pH = 7) were prepared with the potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), magnesium chloride hexahydrate ($MgCl_2(H_2O)_6$), 1 M sodium hydroxide (NaOH), and 6 M hydrochloric acid (HCl). The ammonium carbonate ($(NH_4)_2CO_3$), sodium bicarbonate ($NaHCO_3$), and calcium chloride dihydrate ($CaCl_2(H_2O)_2$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The enzymes pepsin (975 units per protein, porcine), pancreatin (8 × USP specifications, porcine), and porcine bile extract were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Supercritical Fluid Extraction (SFE)

The supercritical fluid extraction (SFE) equipment is located at the ALISOST laboratory at the Faculty of Pharmacy and Food Sciences of the Universitat de València (València, Spain). The SFE extraction process followed a previously established method (Liu et al., 2025a). The parameters

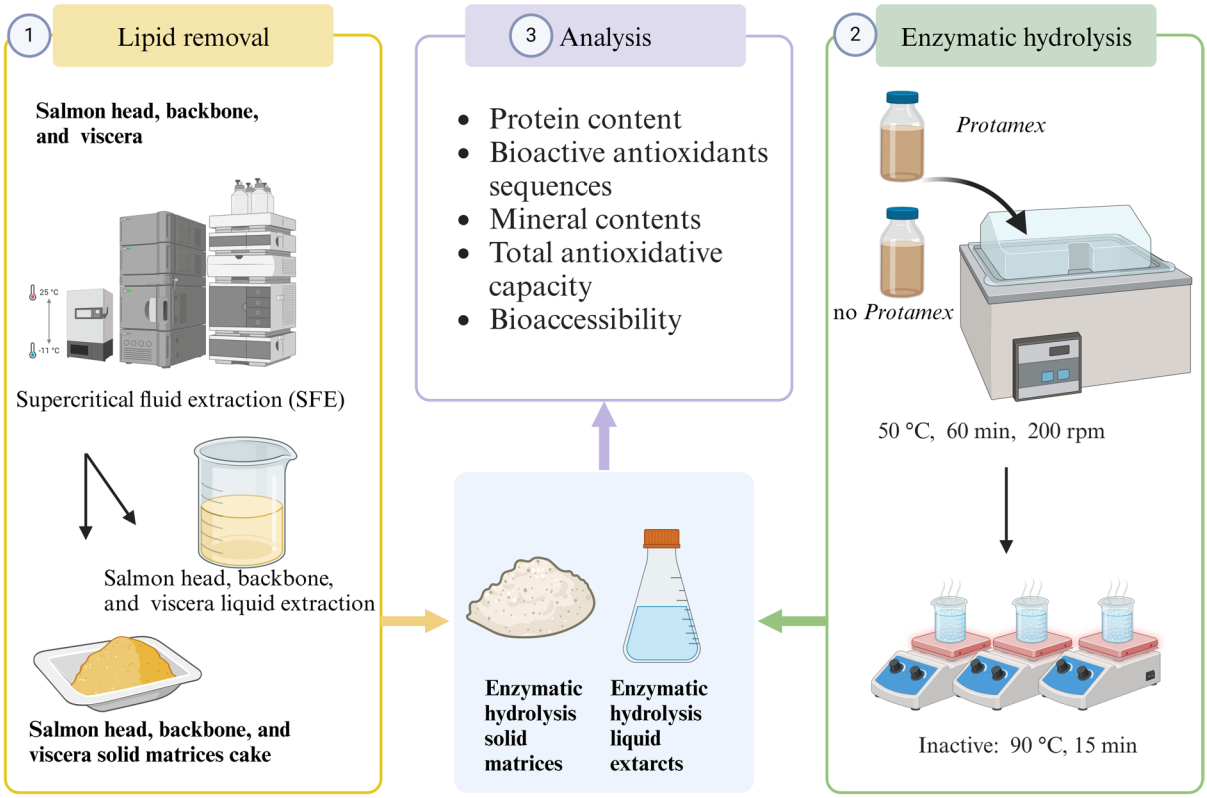


Fig. 1 The lipid-free treatment with supercritical fluid extraction (SFE) and enzymatic hydrolysis (EH) process for salmon side streams (head, backbone, and viscera) (Created in BioRender.com)

included an extraction time of 75.0 min, an extraction temperature of 50.0 °C, and a fluid flow rate of 10 mL/min consisting of 1.0 mL/min absolute ethanol and 9.0 mL/min carbon dioxide (CO_2). Both the solid matrix remaining cakes and the liquid extracts were collected. The solid matrix remaining cakes were then freeze-dried at − 60.0 °C for 48 h to obtain the final solid matrices.

Enzymatic Hydrolysis

Protamex® (protease from *Bacillus* sp.) (Ahn et al., 2014) was used for enzymatic hydrolysis. The hydrolysis conditions were adapted from recent studies with slight modifications (Aspevik et al., 2021; Ma et al., 2021). The parameters used included a hydrolysis temperature of 50.0 °C, a hydrolysis time of 60.0 min, a pH of 7.0, a solid-to-liquid ratio of 1:20 g/mL, and an enzyme addition of 5.0%. The mixture was prepared by combining salmon side streams with distilled water, and the temperature was raised to 50.0 °C before adding the enzyme. After the enzymatic hydrolysis was completed, the enzyme was inactivated by heating the mixture to 90.0 °C for 15 min. The hydrolysate was then centrifuged at 4000 × rpm for 20 min to separate the supernatant from the solid matrices, and the enzymatic hydrolysis liquid extracts. Solid matrices were obtained then followed the − 61.0 °C freeze-dried (Model 10 N, Gedilab S.L., Spain) and then crushed into the power using the Pulverisette 11 (Fritsch, Germany) for 3 min final as the sample to be further analyzed.

Protein and Bioactive Peptide (BP) Analysis Tests

Protein and bioactive peptides were determined according to the method of (Liu et al., 2024). For protein analysis, the weighted dry samples were carefully wrapped and analyzed for nitrogen content using high-temperature combustion in an elemental analysis system. The protein content was then calculated using a nitrogen-to-protein conversion factor of 6.25.

Potential BPs were identified through liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis, performed using a Tims TOF fleX mass spectrometer (Bruker). All the bioactive peptide sequences were determined after the LC–MS/MS analysis, and the specific potential antioxidative for the sequences (probability in 1) was then identified in the online BPs source (BIOPEP-UWM database) according to the previous method described in (Minkiewicz et al., 2019).

Mineral Contents

Samples in both solid matrices and liquid extracts were previously mineralized in a microwave oven (MARS, CEM, Vertex, Spain). Three hundred milligrams of samples, 4 mL HNO_3 (14 M), and 1 mL H_2O_2 (30%v/v) were weighed and added into a digester, and the samples were digested at 800 W, 180 °C for 15 min. The digested samples were taken out and cooled to room temperature. Then, all the samples were filtered after removing nitrogen, and the volume was made up of distilled water. Finally, all the samples were identified and quantified using inductively coupled plasma mass spectrometry (ICP-MS7900, Agilent Technologies, USA) according to the previous method of Liu, Berrada, Wang et al. (2025).

Total Antioxidative Capacity (TAC) Tests

The total antioxidant capacity (TAC) tests were conducted on the protein hydrolysates from liquid extracts. Specifically, both the Trolox equivalent antioxidant capacity (TEAC) and the oxygen radical absorbance capacity (ORAC) assays were performed following previously established methods (de la Fuente et al., 2023).

In Vitro Simulated Gastrointestinal Digestion

A total of 2.5 g of defatted salmon side streams (head, backbone, and viscera) was mixed with 25.0 g of distilled water. The lipid-removed salmon side streams underwent an in vitro digestion process according to the standardized methodology proposed by INFOGEST (Brodkorb et al., 2019), including oral, gastric, and intestinal stages, as outlined below. The procedure followed a previously established method (Wang et al., 2025), including the oral stage, gastric stage, and intestinal stage. Also, the SSF (pH = 7), SGF (pH = 3), and SIF (pH = 7) were prepared prior to the digestion process.

Oral Stage (Chewing) Digestion

In this stage, the α-amylase has not been used. A mixture of 2.5 g of salmon side stream solvent and 2.0 mL of SSF was shaken for 1.0 min. Then, 12.5 μL of $CaCl_2$ was added, followed by distilled water to bring the total volume to 5.0 mL. The mixture was shaken in the water bath at 37 ℃ for 2 min.

Gastric Stage Digestion

In this stage, 4.55 mL of SGF was added to the mixture and vortexed for 1.0 min. Next, 8.0 mg of pepsin (2000 U/mL) derived from porcine gastric tissue and 2.5 μL of $CaCl_2$ were added, followed by another 1.0-min vortex. The pH was adjusted to 3 using 6 M HCl or 1 M NaOH, and distilled water was added to reach a final volume of 10.0 mL. The mixture was incubated in brown bottles in a water bath at 37 °C for 2 h.

Intestinal Stage Digestion

After the gastric stage, 5.5 mL of SIF was added and vortexed for 1.0 min. Subsequently, 2.5 mL of pancreatin (800 U/mL) from porcine pancreas, 1.25 mL of 1.16 M porcine bile extract, and 20.0 μL of $CaCl_2$ were added, followed by another 1.0-min vortex. The pH was adjusted to 7 using 6 M HCl or 1 M NaOH, and distilled water was added to reach a total volume of 20.0 mL. The bottles were incubated in a water bath at 37 °C for 2 h, then centrifuged at 4000*x*rpm for 40 min at 4 °C. The supernatant was collected for mineral content analysis.

The mineral contents were referenced to the mineral content test. The results were expressed as the ratio of the concentration of bioactive compounds in the digested sample to that in the original lipid-removed sample, calculated using the following Eq. (1):

$$\text{Bioaccessibility}(\%) = \left(\frac{\text{content in digesiton supernatants}}{\text{content in lipid removal salmon side streams}}\right) \times 100 \quad (1)$$

Statistical Analysis

All tests were performed in triplicate, with average values reported. Statistical analyses were conducted using one-way analysis of variance (ANOVA) and Duncan's multiple range test to identify significant differences ($P < 0.05$). Graphs were created using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). One-way analysis of variance statistical analyses was carried out with SPSS 20.0 (IBM Corp., Armonk, NY, USA). A three-way ANOVA was performed with GraphPad Prism, and the individual and interactive effects of the three main sources of variation were investigated. Proteomic analysis was conducted at the proteomics facility of SCSIE, University of Valencia, which is a member of Proteored, The Spanish National Institute of Proteomics, which is a proteomic services platform promoted by the Genome Spanish Foundation.

Results and Discussion

Protein Evaluation

The protein contents of solid matrices and liquid extracts obtained from salmon side streams (head, backbone, and viscera) after SFE + enzymatic hydrolysis (EH) treatment

were evaluated and compared with SFE + conventional are shown in Fig. 2. A three-way ANOVA was performed, and the individual and interactive effects of the three main sources of variation of treatment (EH or CE), matrix (solid matrices or liquid extracts), and defatted salmon side streams (head, backbone, and viscera) on the protein content results have been assessed. The three primary factors for the protein content were defatted salmon side streams, matrix, and treatment × matrix, which accounted for 67.6%, 15.3%, and 20.9% of the total variation.

For the head, the protein content ranged from a low of 67.0 g/100 g in the SFE + EH-solid to a high of 93.1 g/100 g in the SFE + EH-liquid. In the backbone, the lowest protein content was 68.7 g/100 g in the SFE + EH-solid, while the highest was 92.9 g/100 g in the SFE + EH-liquid. The viscera exhibited the greatest variation, with the lowest protein content at 30.9 g/100 g in the SFE + EH-solid and the highest at 65.3 g/100 g in the SFE + EH-liquid. It is worth noting that significant differences ($P < 0.05$) were observed in the protein content in liquid extracts depending on the extraction method, resulting in higher content after SFE + EH extraction in comparison with SFE + CE extraction (Fig. 2). This improvement is particularly noticeable in the head and backbone samples.

Similar findings have been reported in previous studies, where innovative extraction techniques enhanced protein yields. For instance, alcalase hydrolysis, optimized using response surface methodology, demonstrated high soluble protein content in fish protein hydrolysates (FPHs) derived from aquaculture turbot (*Scophthalmus maximus*) waste (Vázquez et al., 2020). In that study, the average molecular weight (Mw) of turbot hydrolysates ranged from 1200 to 1669 Da, with the viscera hydrolysates exhibiting the highest content of peptides above 1000 Da and below 200 Da. Similarly, pulsed electric field (PEF) technology has been applied to recover protein content from lipid-removed salmon side streams, showing notable increases in protein levels. This was particularly evident in viscera liquid extracts, which achieved the highest protein content of 67.1 g/100 g (dry weight) (Liu et al., 2025a).

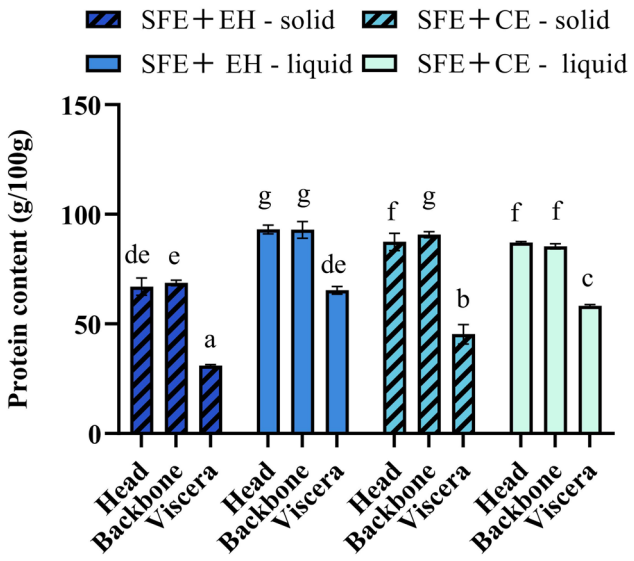


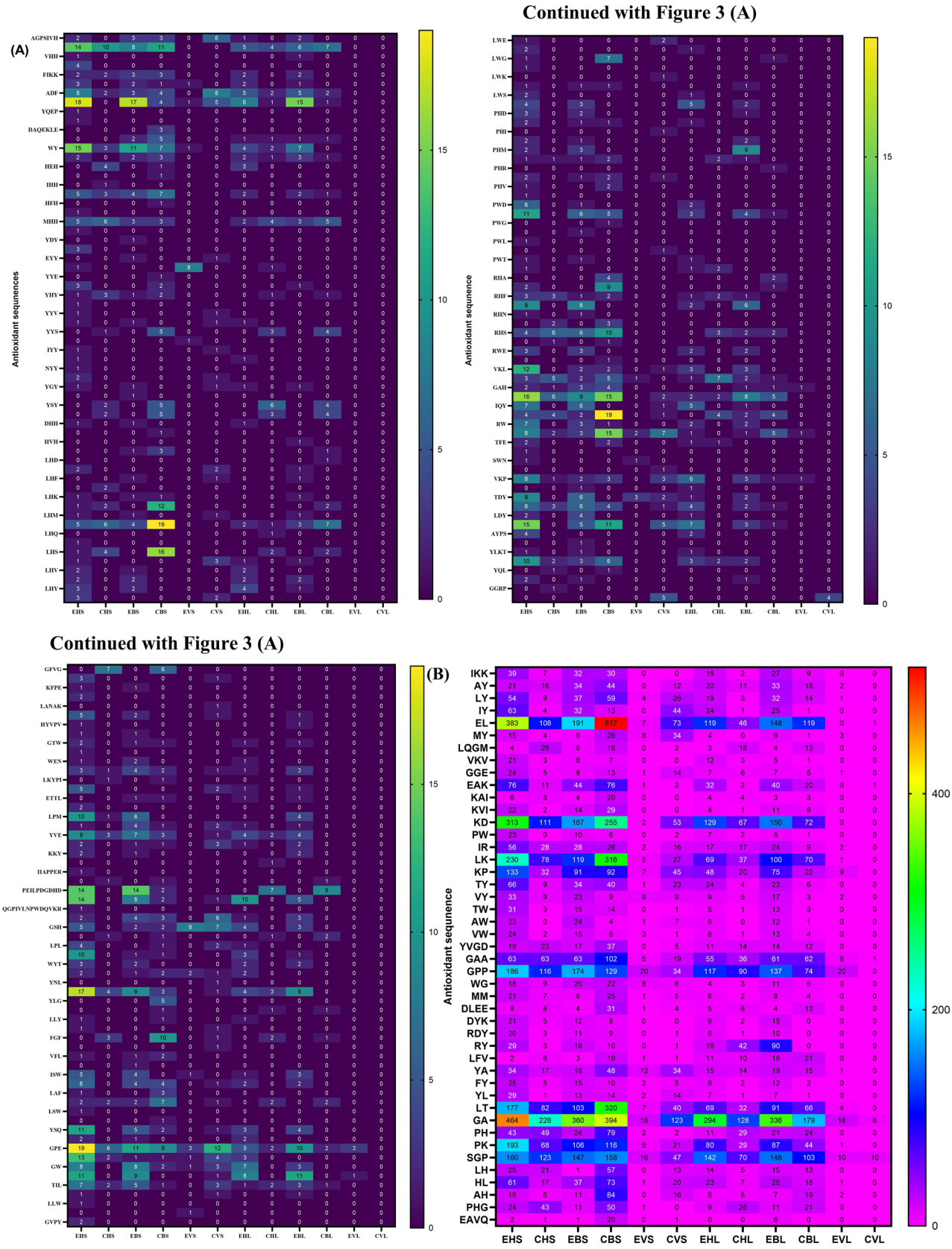
Fig. 2 Protein contents of solid matrices (EH-solid; CE-solid) and liquid extracts (EH-liquid; CE-liquid) obtained from supercritical fluid extraction defatted salmon (head, backbones, and viscera) and subsequent enzymatic hydrolysis (EH) or conventional extraction (CE). Letters show significant differences in the protein content at each extraction of defatted salmon samples (head, backbones, and viscera) (one-way ANOVA and Tukey's HSD test, $P < 0.05$)

Based on these findings, EH holds great potential for future research to optimize protein recovery and explore bioactive peptide sequences. This could lead to the identification of peptides with significant bioactive properties for various applications.

Analysis of BPs

Antioxidant bioactivity plays a crucial role in the potential of bioactive peptides, particularly those derived from fish side streams. In this study, the antioxidant sequences in the solid matrices and liquid extracts obtained from salmon side streams after SFE + EH were analyzed, and they are shown in Fig. 3. Additionally, the frequency of antioxidant sequences identified in each solid matrix and liquid extract from the salmon side streams (head, backbone, and viscera) is summarized in Fig. 4. As it can be seen in the figure, most of these more frequently isolated bioactive peptides are composed of really short chains of amino acids, consisting of 2 to 4 residues. Most of the antioxidant sequences were found in both the EH and CE processes' solid matrices and liquid extracts. However, the solid matrices obtained through the EH contained a greater quantity of antioxidant sequences than those from conventional extraction. These sequences include IKK, AY, LY, IY, EL, MY, LQGM, VKV, GGE, EAK, KAI, KVI, KD, PW, IR, LK, KP, TY, VY, TW, AW, VW, YVGD, GAA, GPP, WG, MM, DLEE, DYK, RDY, RY, LFV, YA, FY, YL, LT, GA, PH, PK, SGP, LH, HL, AH, PHG, and EAVG.

Food-derived BPs, which are protein fragments released from food proteins through physiological treatments, exhibit high potential bioactivity, lower toxicity, smaller molecular weights, easy absorption, and strong targeting capacities (Chang et al., 2025). Similarly, the hydrolysis of sarcoplasmic proteins during the growth of *Lactobacillus plantarum* 120 originally isolated from Suanyu, a traditional Chinese low-salt fermentation fish, was evaluated at 30 ℃ for 96 h (Wang et al., 2017). In that study, there was a pronounced hydrolysis of almost all protein bands in electrophoretic by *L. plantarum* 120 and the generation of new peptides and disappearance of the original can be observed. Moreover,


Continued with Figure 3 (A)
(A)
Antioxidant sequences
AGPSIVH
VIHI
FIKK
ADF
YQEP
DAQEKLE
WY
HEH
IHH
HFH
MHH
YDY
EYY
YYE
YHY
YYV
YYS
IYY
NYY
YGV
YSV
DHH
HVH
LHD
LHF
LHK
LHM
LHQ
LHS
LHV
LHY
EHS CHS EBS CBS EVS CVS EHL CHL EBL CBL EVL CVL
0 5 10 15
Antioxidant sequence
LWE
LWG
LWK
LWS
PHD
PHI
PHM
PHR
PHV
PWD
PWG
PWL
PWT
RHA
RHF
RHN
RHS
RWE
VKL
GAH
IQV
RW
TFE
SWN
VKP
TDY
LDY
AYPS
YLKT
YQL
GGRP
EHS CHS EBS CBS EVS CVS EHL CHL EBL CBL EVL CVL
0 5 10 15
Continued with Figure 3 (A)
Antioxidant sequence
GFVG
KFPE
LANAK
HYVPV
GTW
WEN
LKYPI
ETTL
LPM
YVE
KKY
HAPPER
PEILPDGDHD
QGPIVLNPWDQVKR
GSH
LPL
WYT
YNL
YLG
LLY
FGF
VFL
ISW
LAF
LSW
YSQ
GPE
GW
TIL
LLW
GVPY
EHS CHS EBS CBS EVS CVS EHL CHL EBL CBL EVL CVL
0 5 10 15
(B)
Antioxidant sequence
IKK
AY
LY
IY
EL
MY
LQGM
VKV
GGE
EAK
KAI
KVI
KD
PW
IR
LK
KP
TY
VY
TW
AW
VW
YVGD
GAA
GPP
WG
MM
DLEE
DYK
RDY
RY
LFV
YA
FY
YL
LT
GA
PH
PK
SGP
LH
HL
AH
PHG
EAVQ
EHS CHS EBS CBS EVS CVS EHL CHL EBL CBL EVL CVL
0 200 400

◂**Fig. 3** The antioxidant sequences and frequency of the solid matrices and the liquid extracts obtained from the defatted salmon side streams with EH. 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 (EHS, enzymatic hydrolysis head solid matrices; CHS, conventional extraction head solid matrices; EBS, enzymatic hydrolysis backbone solid matrices; CBS, conventional extraction backbone solid matrices; EVS, enzymatic hydrolysis viscera solid matrices; CVS, conventional extraction viscera solid matrices; EHL, enzymatic hydrolysis head liquid extracts; CHL, conventional extraction head liquid extracts; EBL, enzymatic hydrolysis backbone liquid extracts; CBL, conventional extraction backbone liquid extracts; EVL, enzymatic hydrolysis viscera liquid extracts; CVL, conventional extraction viscera liquid extracts)

greater amounts of free amino acids than the control have been generated during the *L. plantarum* 120 fermentations, especially for alanine, glutamic acid, phenylalanine, valine, methionine, aspartic acid, isoleucine, and leucine. Antioxidative peptides have been reported to perform diverse biological functions, including inhibiting lipid oxidation, neutralizing free radicals, regulating the immune system, delaying aging, and protecting cell membranes. These properties make them widely applicable in the food industry, pharmaceuticals, and health supplements (Chang et al., 2025).

EH, compared to traditional acid or alkaline hydrolysis, provides precise control over peptide bond cleavage while preserving amino acids and enhancing the nutritional value of proteins (Nemati et al., 2024). Furthermore, EH has been shown to maintain or even improve the nutritional value of proteins (Nemati et al., 2024). For example, EH using pepsin and trypsin have been applied to peptides derived from turbot (*Scopthalmus maximus*) viscera hydrolysates. One peptide, Sm-A1 (GITDLRGMLKRLKKMK), demonstrated exceptional antibacterial activity against both Gram-positive and Gram-negative bacteria by disrupting cell membrane integrity (Bi et al., 2020). To maximize the use of Spanish mackerel (*Scomberomorous niphonius*) muscle for antioxidant peptide production, proteins were hydrolyzed using five enzymes and in vitro gastrointestinal digestion, antioxidant peptides were then isolated via ultrafiltration and chromatography (Zhao et al., 2019). In that study, twelve peptides (SMP-1 to SMP-12) were identified, and four peptides including PELDW (Pro-Glu-Leu-Asp-Trp), WPDHW (Trp-Pro-Asp-His-Trp), and FGYDWW (Phe-Gly-Tyr-Asp-Trp-Trp), and YLHFW (Tyr-Leu-His-Phe-Trp) exhibited the higher DPPH radical scavenging activities.

Overall, significant antioxidant bioactivity observed in the SFE + EH of solid matrices and liquid extracts offers valuable insights into peptide activity mechanisms and highlights the potential for targeted delivery of peptides derived from fish side streams. Future studies should also explore other potentially BPs (e.g., anti-inflammatory, antimicrobial, and antihypertensive peptides) and investigate their effects in both in vivo and in vitro.

Analysis of Minerals

The mineral composition of solid matrices and liquid extracts obtained after SFE + EH and SFE + CE were analyzed, and the results are shown in Fig. 4. In general, the solid matrices exhibited higher mineral contents compared to the liquid extracts for magnesium (Mg), phosphorus (P), calcium (Ca), iron (Fe), and zinc (Zn). In contrast, potassium (K) showed higher concentrations in the liquid extracts than in the solid matrices. To better explore the interaction relationship in the mineral results, the three primary sources of variations, namely, treatment (SFE + EH or SFE + CE), matrix (solid matrices or liquid extracts), and lipid-free salmon side streams (head, backbone, and viscera) were applied.

Mg is essential for human bone health and plays a crucial role in protein formation, muscle contraction, immune function, and nerve transmission, and can assist in avoiding constipation (Gharibzahedi & Jafari, 2017). In terms of magnesium (Mg), the three primary factors were the lipid-free salmon side streams × matrix, lipid-free salmon side streams × treatment × matrix, and treatment × matrix, accounting for 66.5%, 12.1%, and 8.4%, of the total variation, respectively. For Mg, the highest and lowest values in the solid matrices were 3.1 mg/g for head-SFE + EH and 0.6 mg/g for viscera-SFE + EH, respectively. SFE + EH retained more Mg in the solid matrices compared to SFE + CE. Mg levels in the head and backbone were significantly higher ($P < 0.05$) than in the viscera. In liquid extracts, the highest Mg value was 2.6 mg/g (viscera-SFE + EH), and the lowest was 1.1 mg/g (backbone-SFE + EH). CE generally yielded higher Mg levels in liquid extracts compared to SFE + EH.

The P is found in every cell, which is needed for the human body to make the protein providing cell growth, maintenance, and reparation (Gharibzahedi & Jafari, 2017). In terms of P, the three primary factors were the matrix, lipid-free salmon side streams, and lipid-free salmon side streams × treatment × matrix, accounting for 27.5%, 27.2%, and 13.6%, of the total variation, respectively. For P in the solid matrices, the highest content (62.5 mg/g) was observed in head-SFE + EH, while the lowest (1.4 mg/g) was found in viscera-SFE + EH. EH retained more P in the solid matrices than SFE + CE, particularly in the head and backbone. Liquid extracts from SFE + CE exhibited a higher P concentration, with the highest value of 4.2 mg/g (backbone-SFE + CE) compared to 5.7 mg/g (head-SFE + EH) which are presented in Fig. 4.

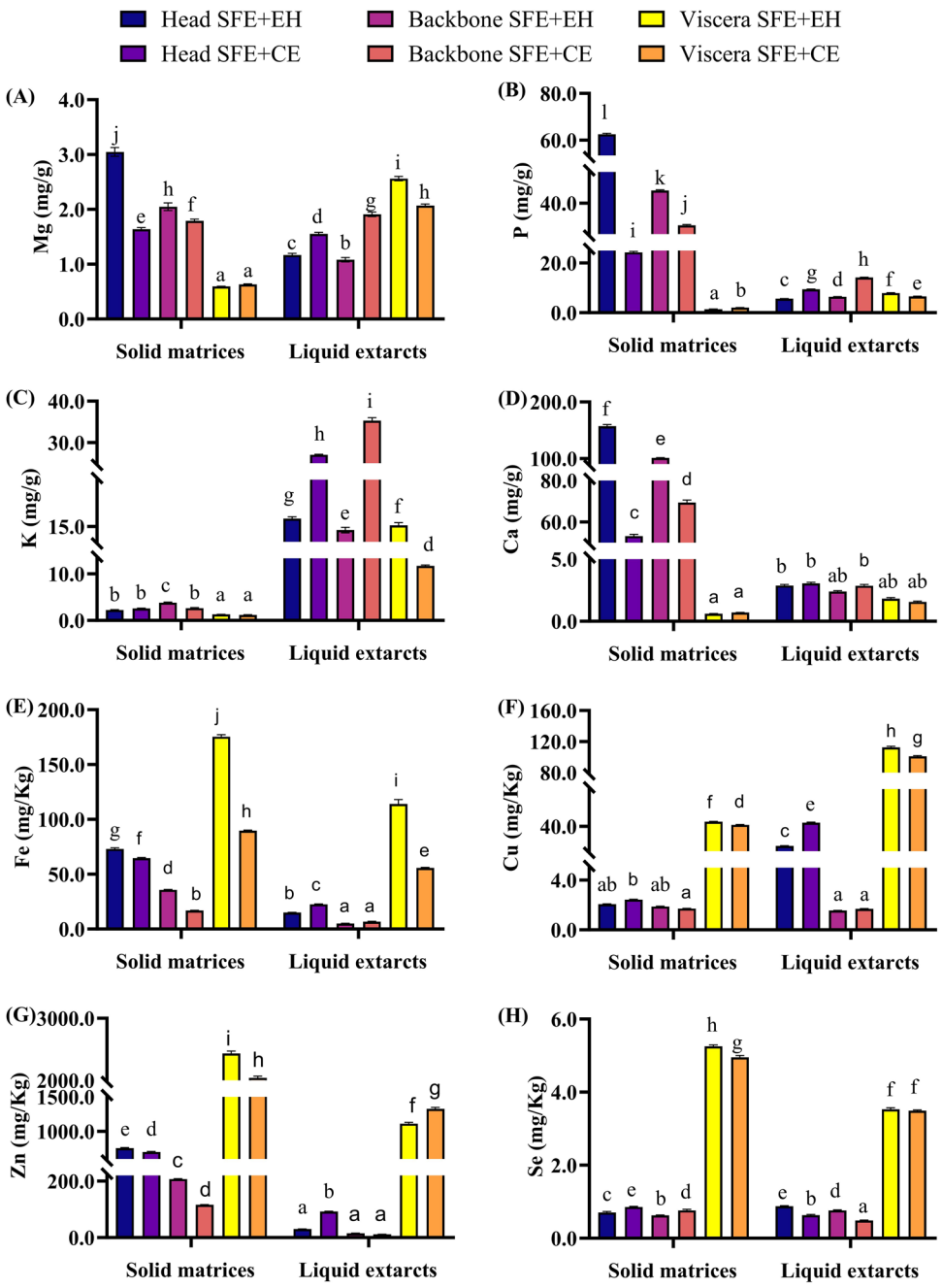


Fig. 4 The mineral content of the solid matrices and the liquid extracts obtained from the Salmon side streams with enzymatic hydrolysis (SFE + EH: supercritical field extraction + enzymatic hydrolysis; SFE + CE: supercritical field extraction + conventional extraction)

In terms of K, the three primary factors were the matrix, lipid-free salmon side streams, and lipid-free salmon side streams × treatment × matrix, accounting for 68.5%, 7.1%, and 5.8% of the total variation, respectively. For K, the solid matrices showed a range of 1.2–3.8 mg/g, with significantly higher levels in backbone-SFE + EH compared to SFE + CE. In liquid extracts, K levels ranged from 11.7 mg/g (viscera-SFE + CE) to 35.3 mg/g (backbone-SFE + CE). Interestingly, EH significantly enhanced ($P < 0.05$) K extraction from viscera into the liquid phase.

In terms of calcium (Ca), the three primary factors were the matrix, lipid-free salmon side streams, and lipid-free salmon side streams × matrix, accounting for 38.2%, 21.4%, and 20.4% of the total variation, respectively. In terms of the Ca, the solid matrices displayed values between 0.6 mg/g (lowest) and 157.0 mg/g (highest), with SFE + EH retaining more Ca in the head and backbone than SFE + CE. Liquid extracts showed Ca levels between 1.6 and 3.1 mg/g.

In terms of iron (Fe), the three primary factors were the lipid-free salmon side streams, matrix, and lipid-free salmon side streams × treatment, accounting for 62.5%, 16.1%, and 10.6% of the total variation, respectively. Iron (Fe) is an essential component of various enzymes, including cytochromes, hemoglobin, and myoglobin. It plays a crucial

role in biochemical processes such as energy metabolism, substrate utilization, and the transport of oxygen and electrons (Shubham et al., 2020). As for the Fe, the highest value (175.4 mg/Kg) and lowest values (16.9 mg/Kg) in solid matrices and the highest values (114.0 mg/Kg) and lowest values (5.1 mg/Kg) in liquid extracts have been presented.

In terms of Cu, the three primary factors were the lipid-free salmon side streams, matrix, and lipid-free salmon side streams × matrix, accounting for 71.0%, 16.1%, and 9.0% of the total variation, respectively. As for the Cu, the highest value (43.7 mg/Kg) and lowest values (1.7 mg/Kg) in solid matrices and the highest values (101.0 mg/Kg) and lowest values (1.6 mg/Kg) in liquid extracts have been presented. As for the Zn, the highest value (2433.0 mg/Kg) and lowest values (115.9 mg/Kg) in solid matrices and the highest values (1327.0 mg/Kg) and lowest values (11.4 mg/Kg) in liquid extracts have been presented. As can be seen in Fig. 4, for solid matrices and liquid extracts, the Cu contents in the viscera are significantly higher than the values in the head and backbones.

In terms of Zn, the three primary factors were the lipid-free salmon side streams, matrix, and lipid-free salmon side streams × matrix, accounting for 79.0%, 14.6%, and 5.0% of the total variation, respectively. As for Zn, the highest values (2433.0 mg/Kg) and lowest values (115.9 mg/Kg) in the solid matrices, and the highest values (1112.0 mg/Kg) and lowest values (11.4 mg/Kg) in the liquid extracts can be observed in Fig. 4. Moreover, a significant difference can be observed that most of the Zn is determined in the viscera compared to the head and backbone.

In terms of Se, the three primary factors were the lipid-free salmon side streams, lipid-free salmon side streams × matrix, and matrix, accounting for 92.2%, 4.6%, and 2.7% of the total variation, respectively. As for the Se, the highest value (5.3 mg/Kg) and lowest values (0.6 mg/Kg) in solid matrices. Lesser Se contents can be determined in the head and backbone. Also, a large quantity of Se is presented in the viscera. Liquid extracts exhibited Se levels between 0.6 and 3.5 mg/kg, with SFE + EH showing a greater ability to extract Se from the solid matrices into the liquid phase compared to SFE + CE. Hence, the EH might have the ability to promote the Se from the sea bass side streams' cake into the liquid extracts.

Our relative research has claimed that fish with a high content can not only be applied as dietary mineral supplements but also have the potential to promote the development of the fish processing industry and reduce fish waste for the environment (Wang et al., 2025). In this study, the primary source of variation is the matrix for P, K, and Ca, and the primary source of variation is the lipid-free salmon side streams for Fe, Cu, Zn, and Se. Therefore, the matrices and types of lipid-free salmon side streams can significantly affect the extraction of minerals after SFE + EH. Overall, these results indicate that SFE + EH is effective in retaining higher mineral concentrations in solid matrices, while also promoting the extraction of certain minerals, such as Se and K, into the liquid extracts. This demonstrates the potential of SFE + EH for enhancing mineral recovery from fish side streams.

Analysis of TAC

As shown in Fig. 5A, B, the TAC was evaluated using the TEAC and ORAC assays, revealing a significant increase in antioxidant activity following enzymatic hydrolysis compared to the control.

For the salmon head and backbone, a similar trend was observed in both TEAC and ORAC assays, where enzymatic hydrolysis consistently yielded significantly higher values than the control. In the TEAC assay, the antioxidant capacities were as follows: 160.4 μM Trolox (head SFE + CE), 1715.8 μM Trolox (head SFE + EH), 239.0 μM Trolox (backbone SFE + CE), and 1822.5 μM Trolox (backbone SFE + EH). In the ORAC assay, the results were 2273.7 μM Trolox (head SFE + CE), 8628.3 μM Trolox (head SFE + EH), 2660.8 μM Trolox (backbone SFE + CE), and

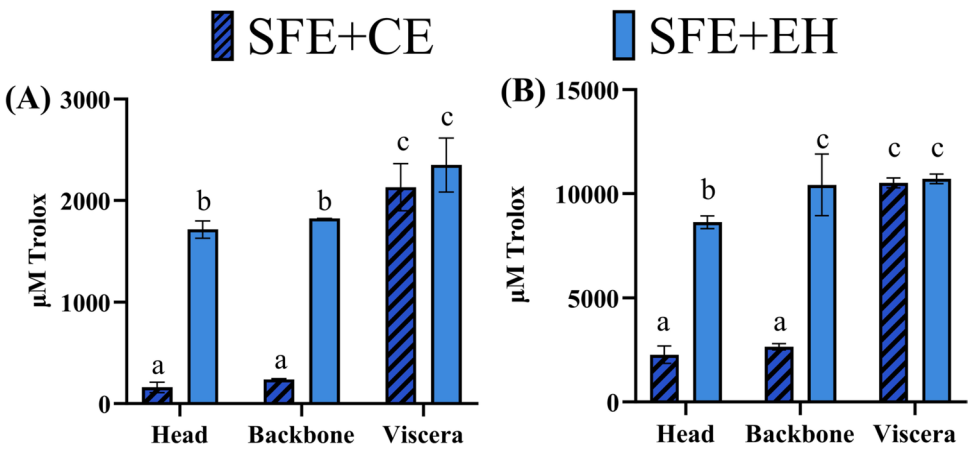


Fig. 5 The total antioxidant capacity (TAC) of the liquid extracts obtained from defatted salmon side streams with enzymatic hydrolysis. **A** TEAC and **B** ORAC

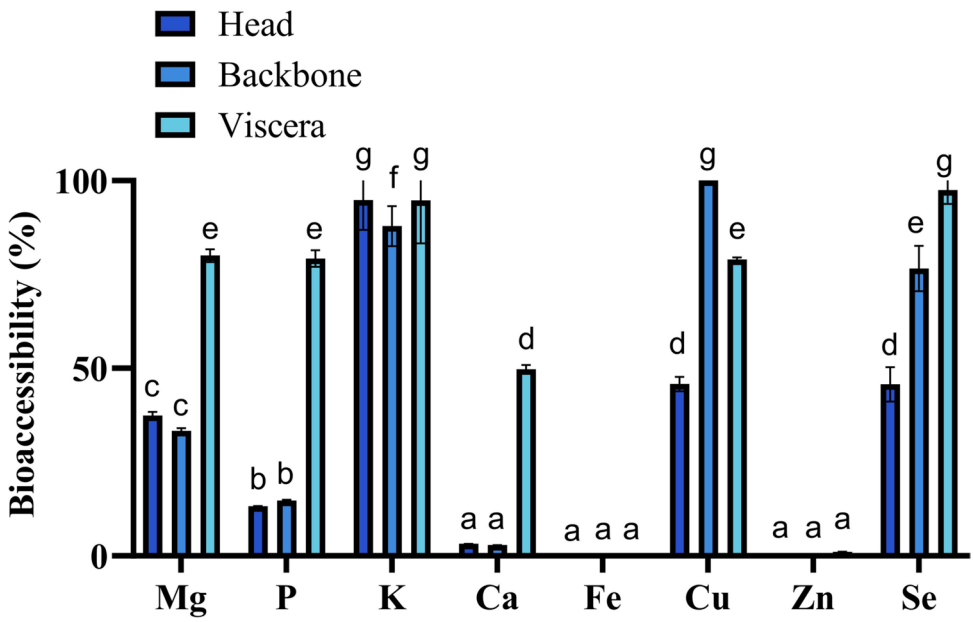


Fig. 6 The mineral bioaccessibility of defatted salmon side streams (head, backbone, and viscera) after in vitro simulated gastrointestinal digestion

10,424.4 μM Trolox (backbone SFE + EH). These findings highlight the substantial enhancement of total antioxidant capacity by EH compared to CE.

Interestingly, the salmon viscera exhibited the highest antioxidant capacity among all the side streams analyzed. In the TEAC assay, the antioxidant values were 2131.7 μM Trolox for the SFE + CE and 2350.4 μM Trolox for SFE + EH. Similarly, in the ORAC assay, the results were 10,521.5 μM Trolox for the SFE + CE and 10,713.6 μM Trolox for SFE + EH.

A related study investigated the antioxidant capacity of enzymatic hydrolysates derived from low-valued ray-finned fish (*Labeobarbus nedgia*), assessing DPPH and ABTS radical scavenging capacities as well as ferric reducing antioxidant power (Tadesse et al., 2023). All enzymatic hydrolysates demonstrated antioxidant activity, reinforcing the effectiveness of enzymatic hydrolysis in enhancing antioxidant potential. Hence, the results in this study suggest that enzymatic hydrolysis is highly effective in boosting the antioxidant potential of salmon side streams, aligning with findings from previous research, which also reported enhanced antioxidant activity through innovative extraction techniques.

Mineral Bioaccessibility

As can be observed in Fig. 6, the mineral bioaccessibility for lipid-free salmon head, backbone, and viscera samples was investigated, and we found significant differences ($P < 0.05$) among the various salmon side streams. The bioaccessibility of minerals Mg, P, K, Ca, Fe, Cu, Zn, and Se ranged from 0.1 to 100.0%, and the highest bioaccessibility has been presented in the mineral Cu (100% for backbone) and K (94.8% for head). In contrast, the lowest bioaccessibility was displayed in the mineral Fe, with a value of 0.1% for the lipid-free salmon viscera. Also, the second-lowest bioaccessibility is in the mineral Zn, which was 0.2% for the lipid-free salmon backbone.

Previous studies have extensively investigated mineral bioaccessibility in fish side streams to examine heavy metals, and mineral bioaccessibility in rainbow trout (*Oncorhynchus mykiss*) and Dover sole (*Solea solea*) side stream extracts obtained through pressurized liquid extraction (PLE) (Wang et al., 2023). The findings revealed that PLE significantly enhanced the mineral content in the extracts (up to sixfold). However, PLE did not uniformly improve the bioaccessibility of all minerals, as the recovery rates and bioaccessibility varied depending on the type of fish and specific minerals analyzed. Similarity, the bioaccessibility of minerals in the salmon (*Salmon salar*) and mackerel (*Scomber scombrus*) backbone and head has also been explored (de la Fuente et al., 2023). The highest mineral bioaccessibility was observed in salmon and mackerel head hydrolysates for Fe (≥ 100%), followed by Se in salmon backbone hydrolysate, which exhibited a bioaccessibility rate of 95%. Additionally, recent research investigated the digestibility of fish skin-derived protein hydrolysates before and after in vitro simulated digestion (Wang et al., 2025). Results showed that white fish protein exhibited high digestibility (up to 92%), while salmon collagen had the highest digestibility (73%) among collagen samples.

Therefore, these findings highlight the significant variation in mineral bioaccessibility among fish side streams and reinforce the effectiveness of enzymatic and extraction techniques in enhancing mineral content and digestibility.

Conclusions

This study aims to explore both the potential bioactive compounds, and the economic benefits of lipid-free salmon side streams derived from the EH and CE process. Specifically, the protein content, BPs, and mineral composition of both solid matrices and liquid extracts, as well as the TAC of the liquid extracts, have been examined. Our findings indicate that EH and CE significantly enhanced protein contents in liquid extracts, particularly from lipid-free salmon side streams. The analysis reveals that protein levels are significantly higher in the liquid extracts (EH and CE), demonstrating the effectiveness of these processes in extracting proteins from salmon by-products. Regarding bioactive antioxidant capacity, the solid matrices from the head and backbone (following EH and CE) exhibit significantly higher antioxidant potential. Notably, key bioactive peptides such as EL (glutamic acid-lysine), GA (glycine-alanine), LT (leucine-threonine), LK (leucine–lysine), and KD (lysine-aspartic acid) are found in substantial quantities. The mineral content of both solid matrices and liquid extracts follows distinct trends. Particularly, salmon viscera contain higher levels of essential minerals such as Fe, Cu, Zn, and Se compared to the head and backbone. The mineral levels can be significantly affected by the matrices and the lipid-free salmon side streams according to the three-way ANOVA analysis. Additionally, the bioaccessibility of minerals in lipid-free salmon side streams treated with SFE ranges from 0.1 to 100.0%. Overall, the use of EH facilitated the recovery of high-added-value compounds, including proteins, bioactive peptides, and minerals, contributing to the TAC. These findings align with results from sequential extraction methods using SFE and pulsed electric fields (PEF), which have also been shown to enhance the recovery of bioactive compounds and minerals in salmon side streams (Liu et al., 2025a). This study advances salmon side stream valorization by demonstrating that EH outperforms CE in recovering proteins, bioactive peptides, and minerals from SC-CO_2-defatted materials, while revealing key differences in antioxidant distribution and mineral bioaccessibility. The findings establish EH as a superior, sustainable approach for transforming processing byproducts into high-value nutraceutical and food ingredients, while identifying optimization targets for industrial-scale applications. Moving forward, we will focus on protein hydrolysate techniques and their applications, presenting them as a foundation for further research, education, and industrial-scale utilization. Future studies could also explore the combined use of EH with PEF to further optimize the extraction of high-added-value compounds. The combined SFE and EH approach is not limited to salmon side streams, it can also be applied to other enzymes, other fish processing residues, and marine biomass (e.g., *spirulina*) for bioactive compound recovery. While this study demonstrates the method's effectiveness, future research should focus on scaling up the process from lab to pilot and industrial systems, addressing technical, economic, and processing challenges.

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

Declarations

Competing interests The authors declare no competing interests.

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