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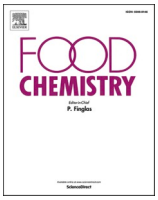

High-added-value compounds recovery from supercritical fluid extraction-defatted European sea bass (*Dicentrarchus labrax*) viscera by lactic acid bacteria (*L. plantarum* and L. *casei*) assisted fermentation

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ABSTRACT

In this study, supercritical-CO_2 (25.0 MPa, 40 °C, 75 min, 10.0 mL/min) was used to defeat sea bass viscera to facilitate the fermentation process. Then, the defatted samples were used as raw material to be fermented by lactic acid bacteria (*Lactobacillus plantarum* and *Lactobacillus casei*) over 72 h. After fermentation, solid matrices and supernatants were separated by centrifugation and analyzed for protein content, bioactive peptides, and minerals. Both LAB strains significantly ($P < 0.05$) increased protein content (24.9 % and 27.4 %) in the supernatants. Six bioactive peptides were identified, demonstrating antioxidants, anti-inflammatory, anticancer, antithrombotic, immunomodulatory, and hypolipidemic properties. Some 28 antioxidant peptide sequences were found, especially for EL (glutamic acid-phenylalanine) and KD (lysine-aspartic acid). Moreover, the mineral results showed the possibility of mineral accessibility, while the LAB strains also notably enhanced the antioxidants of the supernatants. This study highlights the potential of fish side streams in generating bioactive compounds through fermentation.

1. Introduction

Fish side streams, such as viscera, heads, and backbones, have been traditionally discarded by the fish industry due to their strong odors and microbial load, thus hindering their subsequent utilization as food. Protein-rich fish processing side streams, often called rest raw materials (RRM), account for approximately 60 % of the total fish biomass (Kaushik et al., 2024). However, these fish processing side streams are actually rich sources of nutrients, making them excellent raw materials for animal feed preparation (Sandström et al., 2022). These findings indicate a paradigm shift in fish processing, which might be moving beyond the traditional role of food production toward the development of functional foods and nutraceuticals with significant health-promoting properties (Kurnianto et al., 2024). Therefore, it is essential to adopt new approaches to utilize these fish side streams as valuable resources for food products and even health applications development. Hence, fermentation has emerged as a method with a big potential to address this significant challenge (Marti-Quijal et al., 2020).

Fermentation has been used since ancient times for food applications, being considered one of the oldest and most widely used food preservation techniques. Moreover, it can promote flavor enhancement, nutrient enrichment, cost-effectiveness, and straightforward operation flavors, among others (Cai et al., 2024). This process often involves the use of starter cultures which naturally promote and dominate the fermentation of various foods (Marcelli et al., 2024). These cultures are generally recognized as safe (GRAS) and carry Qualified Presumption of Safety (QPS) status, acknowledged by both the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) (Marcelli et al., 2024). *Lactobacillus* species are the most common species among all the lactic acid bacteria (LAB) genera, which have an important influence on the physicochemical properties of fish sauces during fermentation (Han et al., 2023). LAB, such as *Lactobacillus plantarum* (*L. plantarum*) (Chen et al., 2024) and *Lactobacillus casei* (*L. casei*) (Maciel da Silva et al., 2022), play a crucial role in the fermentation process as

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they metabolize carbohydrates into lactic acid, which lowers pH levels, enhancing food safety (Cai et al., 2024).

Fermented fish products are well-known globally, with varieties such as fermented fish (Prihanto et al., 2024) and fermented golden pompano (*Trachinotus ovatus*) being particularly popular in Asia and other regions (Y. Wang et al., 2024).

Under the influence of bacterial enzymes and endogenous enzymes, raw materials undergo carbohydrate fermentation, amino acid degradation, lipid oxidation, and other transformations that yield essential fatty acids, amino acids, and other primary metabolites (Fitri et al., 2022). The distinctive flavor of fermented fish products results from a complex interplay of metabolic reactions by diverse microbiota during fermentation, which includes carbohydrate fermentation, protein degradation, and fat oxidation (Han et al., 2023). These primary metabolites promote microbial growth, leading to the formation of volatile secondary metabolites, which promotes the formation of different flavors, aromas, and textures to the final product (Zhang et al., 2023).

Lipid-free protein hydrolysates from fermented fish products can be also commercially valuable and contain some potential bioactivities, such as the ACE-I inhibitory activity, which can serve as a natural alternative to synthetic drugs for hypertension (Yathisha et al., 2023). The lipid removal step can enhance the protein content and analyze the physicochemical and bioactive properties during the extraction process (such as lipase enzyme (Yathisha et al., 2023), pulsed electric fields (PEF) (Liu et al., 2025), and supercritical fluid extraction (SFE) (Liu, Berrada, Pallarés, et al., 2024)). Therefore, there is a need for innovative, sustainable, and green approaches like supercritical fluid extraction (SFE) to remove lipids from sea bass viscera and to get defatted samples.

Considering the aforementioned, this research aims to evaluate the nutrient profile of lipid-free sea bass viscera following lactic acid fermentation to understand if it can constitute a promising method for recovering high-added-value compounds from fish side streams. Specifically, this was the first attempt to combine two safe, low energy consuming, and environment-friendly techniques such as SFE and fermentation to promote the high-added-value compounds´ recovery from fish side streams.

For this purpose, this study utilized defatted sea bass viscera as a fermentation broth, with L. *plantarum* and L. *casei* as the fermentation strains. Throughout the fermentation process, bacterial counts and pH levels were measured every 24 h to monitor progress. After fermentation, solid matrices and supernatants were collected separately and subjected to centrifugation. Both the solid and liquid fractions were analyzed for protein content, bioactive peptides, minerals, and heavy metals. Additionally, the antioxidant capacity of the supernatants was assessed using TEAC and ORAC assays.

2. Materials and methods

2.1. Lactic acid bacteria and fish side streams (viscera)

The lactic acid bacteria strains (*Lactobacillus plantarum* and *Lactobacillus casei*) were obtained from the Spanish Type Culture Collection (CECT) in Valencia, Spain. As the only public Microbial Biological Resource Centre (mBRC) in Spain, the CECT serves as a repository and provider of various microorganisms, including bacteria, yeast, filamentous fungi, and archaea. Dead sea basses (*Dicentrarchus labrax*), preserved on ice, were purchased from a supermarket in Valencia, Spain. The sea bass visceras were then prepared in the laboratory. After obtaining the viscera, they were freeze-dried for 72 h and subsequently crushed into small pieces. The pulverization process was carried out using a Pulverisette 11 (Fritsch, industries. 855,743 Idar-operateln, Germany) set to 220–240 V, 50/60 Hz, and 600 W for 3 min. The

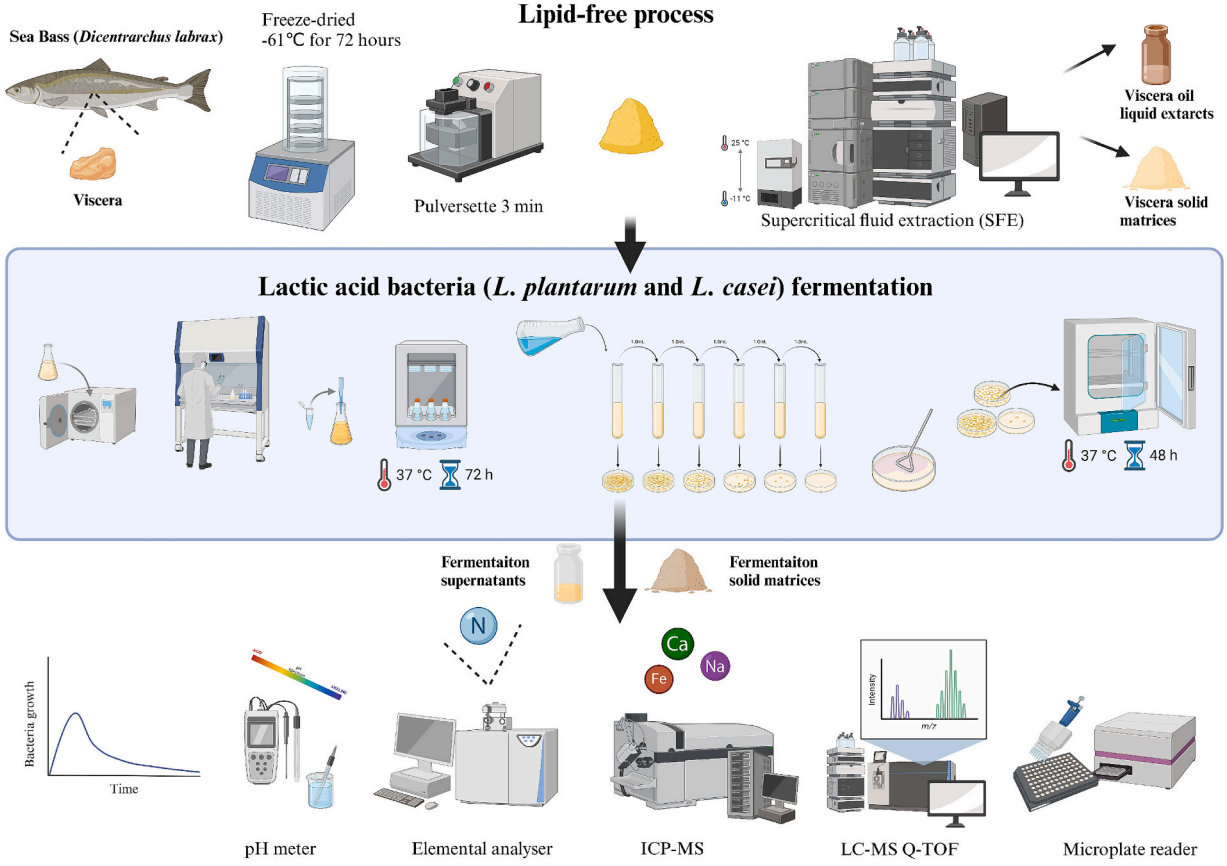

Fig. 1. Schematic representation of the supercritical fluid extraction to get the lipid-free sea bass viscera and subsequent lactic acid bacteria fermentation process to recover the high-added-value compounds (Created in BioRender.com).

processed viscera were then stored at −20 °C until further use. The entire pretreatment and fermentation process for the sea bass viscera is outlined in Figure 1.

2.2. Chemicals and reagents

MRS agar and MRS broth were purchased from Condalab (Laboratories Conda S.A.). 6-hydroxy-2,5,7,8- tetramethyl chroman-2-carboxylic acid (Trolox), potassium persulfate ($K_2S_2O_8$) was purchased from the Sigma-Aldrich (Steinheim, Baden-Württemberg, Germany). 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was purchased from ThermoFisher (Kandel, Germany). Deionized water was obtained by a Milli-Q SP Reagent Water System (Millipore Corporation, Bedford, MA, USA).

2.3. Supercritical fluid extraction (SFE) extraction

The SFE process was performed using the supercritical fluid extraction located at the laboratory of the ALISOST team at the Faculty of Pharmacy and Food Sciences (Universitat de València, València, Spain) as the previous extraction aimed to remove the lipids compounds from the sea bass viscera (dry weight, d. w), following protocols established in previous research by our team. After the SFE process (40.0 °C, 25.0 MPa, in dynamic extraction in 10.0 mL/min (9.0 mL/min of CO_2 and the 1.0 mL/min of ethanol absolute) for 75 min), the defatted sea bass viscera were freeze-dried for 72 h and then stored at −20 °C until ready for use in the fermentation process.

2.4. Lactic acid bacteria fermentation

The sea bass viscera broth was prepared with 1.5 g of viscera solid form sea bass (dry weight) and 30 g of deionized water, then sterilized in an autoclave at 95 °C for 20 min to prevent microorganism contamination (Bezerra & Fonseca, 2023). After the sterilization, the viscera broths were allowed to cool to room temperature before use. To carry out the fermentation, *L. plantarum,* and L. *casei* were selected as lactic acid bacteria strains and incubaiton lactic acid bacteria contention was controlled in the OD_{600} values between 1.5 and 1.8. Then, 300 μL of the bacterial culture (accounting for 20 % of the total solid weight) was added to the viscera broth. The fermentation process was carried out at 37 °C for 72 h. Bacterial counts and pH levels were measured on MRS agar plates at intervals of 0, 24, 48, and 72 h.

Following fermentation, the sea bass viscera broths were centrifuged at 4000*x*rpm for 30 min to separate the supernatants and solid matrices. Half of the supernatants and solid matrices were then freeze-dried at −50 °C for 48 h and subsequently pulverized for analysis. The resulting powders were used to assess protein content, bioactive peptides, mineral content, and heavy metal levels.

2.5. Bacteria count and the pH determination during the fermentation process

To monitor bacterial growth throughout the fermentation process, 100 μL of the sea bass viscera broth was inoculated onto MRS agar plates at intervals of 0, 24, 48, and 72 h to determine lactic acid bacteria counts.

Additionally, pH measurements were taken to track changes during fermentation using the FP20 Five Easy Plus pH meter (Mettler-Toledo, LLC, United States). This equipment provided accurate pH readings, essential for assessing the activity of the lactic acid bacteria strains, L. *plantarum* and *L. casei*, over the entire fermentation period.

2.6. Protein content determination

The protein content of the dry weight power samples (fermentation supernatant and the solid matrices) was determined with the Dumas method (DW) (Serrano et al., 2013) with the EuroVectoer Elemental Analyser (EuroVector SpA, Milan, Italy). The protein content was then calculated using a nitrogen-to-protein conversion factor of 6.25, which is appropriate for fish and fish sidestreams (Liu, Berrada, et al., 2024; Serrano et al., 2013).

2.7. Bioactive peptides determination

The potential bioactive peptide activities were investigated both for the fermented supernatants and the solid matrices. To determine the bioactive peptides profile, a liquid chromatography-tandem mass spectrometry (LC-MS) Q-TOF system (6600plus Triple TOF, ABSCIEX, Framingham, MA, USA) was used according to the method described by (Liu, Berrada, Wang et al., 2024). Then, the peptide sequences were referenced according to the BIOPEP-UWM databases to isolate the specific potential peptide activities (Minkiewicz et al., 2019).

2.8. Inductively coupled plasma spectrometer detector (ICP-MS) determination of mineral and heavy metals

The ICP-MS (ICP-MS7900, Agilent Technologies, Santa Clara, CA, USA) equipment was to determine the minerals and heavy metals profiles of the samples evaluated. The samples were mineralized in the microwave oven, digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2), then the nitrogen was removed. All the ICP-MS determination process was carried out according to (M. Wang et al., 2023).

2.9. Total antioxidant capacity determination

2.9.1. Trolox equivalent antioxidant capacity (TEAC) assay

According to the previous methods (M. Wang et al., 2021), the TEAC was performed with UV spectroscopy (PerkinElmer). The mother solution was prepared with the 25 mL ABTS (7 mM) and 440 L $K_2S_2O_8$ (140 mM) following stored in the darkness for 16 h. This solution was diluted to create a working solution. For the assay, 2.5 mL of the working solution was combined with 100 μL of appropriately diluted samples, and absorbance was measured after a 3-min reaction period. The antioxidant capacity was calculated using a standard curve.

2.9.2. Oxygen radical absorbance capacity (ORAC) assay

The ORAC was carried out to assess the antioxidant compounds to scavenge peroxyl radicals according to previous methods (M. Wang et al., 2021). The microplate reader (FLUOstar Omega, Bmg Labtech, German) was utilized with fluorescence during the excitation wavelength of 485 nm and emission wavelength of 535 nm, and the whole temperature was kept at 37.0 °C. The phosphate working buffer (pH between 7.0 and 7.4) was prepared with sodium phosphate dibasic solvents, potassium dihydrogen phosphate solvents, and Milli-Q water. Trolox (100 μM) and fluorescein solutions were also prepared using the phosphate buffer. In a 96-well plate, 50.0 μL of fluorescein and 50.0 μL of either Trolox or sample solution were mixed and incubated in the dark at 37 °C for 10 min. Then, 50.0 μL of AAPH solution was added to initiate the reaction, and fluorescence measurements were taken accordingly.

2.10. Statistical analysis

All tests were conducted in triplicate, and average values are presented. A one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to identify significant differences ($P < 0.05$). Statistical analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA), while GraphPad Prism (GraphPad Software, La Jolla, CA, USA) was used for creating graphs. The bioactivity sequence of determination sequenced has been isolated according to the BIOPEP-UWM databases (Minkiewicz et al., 2019).

3. Results and discussion

3.1. Bacteria counting and the pH changes during the fermentation process

Bacteria growth counting and the acidification changes (Cai et al., 2024) play a significant role in the fish viscera fermentation, as they directly present the status of the lactic acid bacteria growth. Lactic acid bacteria counts were measured during the fermentation process in the 0, 24.0, 48.0, and 72.0 h. As can be observed in Fig. 2, both the L. *plantarum* and L. *casei* significantly promote the highest bacteria counting after 24 h compared with the other incubation times, being this enhancement considerably higher ($P < 0.05$) for the L. *plantarum*. Following this period, a marked ($P < 0.05$) decrease in both L. *plantarum* and *L. casei* growth was noted from 24 to 48 h. As for the control, there are no lactic acid bacteria that were added to the sea bass viscera broth, whereas there is a pH change that can be observed between 0 and 72 h.

These findings suggest that the first 24 h of fermentation are the most critical for enhancing the growth of L. *plantarum* and *L. casei* in sea bass viscera broth. One reason may be that the viscera broth contains a high content of protein and is not satisfied with the lactic acid bacteria growth for a long time. These results agree with some previous findings of our research team after fermentation of sea bass meat and side streams broths assisted by different lactic acid bacteria (Martí-Quijal et al., 2020). The results demonstrated that stomach bacteria had the highest growth in the by-products broth (WB) after 72 h of incubation, a growth from 6 to 11, 6–13.6, and 9–12 log CFU/mL occurred for bacteria from the colon, stomach, and intestine, respectively.

As illustrated in Fig. 3, the pH values decreased with the elapse of fermentation time for all samples. For instance, for L. *plantarum* a significant decrease ($P < 0.05$) (from 5.4 to 5.1.) was found after the 24-h fermentation, while pH values decreased from 5.4 to 5.0 for L. *casei*. This significant pH reduction in the first 24 h correlates with the peak in bacterial counts shown in Fig. 2. Similar pH decline over the 72-h fermentation period has been observed in previous studies on sea bass side streams fermented with lactic acid bacteria (Martí-Quijal et al., 2020).

3.2. Protein content

Protein is a crucial component in raw materials, playing a key role in developing the flavor of fish products during fermentation and protein hydrolysis is a significant biochemical reaction that occurs during this process (Wang et al., 2024). Protein content is not only critical to the overall quality of food products but is also a focal point for consumer interest due to its influence on nutritional profiles. Changes in protein content may occur following fermentation. Fig. 4 presents the protein content of both the solid matrices and supernatants obtained from the sea bass viscera broth fermentation process.

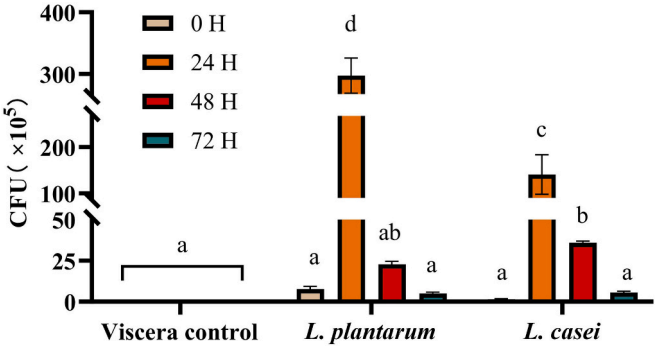

Fig. 2. The lactic acid bacteria counting during the fermentation process with the sea bass viscera broth.

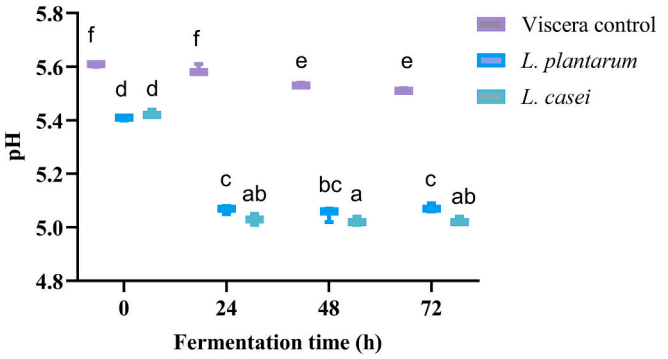

Fig. 3. Changes in pH values during the fermentation process with the sea bass viscera broth.

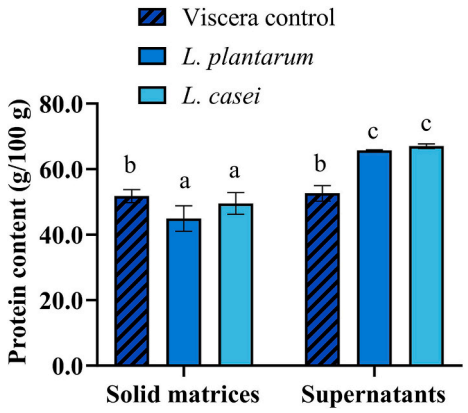

Fig. 4. The protein contents of fermentation solid matrices and supernatants obtained after the fermentation process with the sea bass viscera broth.

The protein contents in fermentation solid matrices (51.8 g/100 g for viscera control, 44.9 g/100 g for L. *plantarum*, and 49.5 g/100 g for L. *casei*) and fermentation supernatants (52.6 g/100 g for viscera control, 65.7 g/100 g for *L. plantarum*, and 67.0 g/100 g for *L. casei*) can be observed in Fig. 4**.** It is evident that lactic acid bacteria (L. *plantarum* and L. *casei*) significantly enhanced the whole fermentation process. As for the viscera control, no significant differences were found in the protein content between solid matrices and supernatants. Whereas the protein contents in fermentation with L. *plantarum* and *L. casei* have a significant increase ($P < 0.05$) in the protein content of the fermented supernatants observed compared to the fermented solid matrices. Furthermore, fermentation-assisted with L. *casei* yielded higher protein values (67.0 %) in supernatants compared to L. *plantarum* (65.7 %).

Previous studies have suggested that protein aggregates are associated with amino acid side chains and protein aggregates (Carbonaro et al., 2012). Previous fermentation researches indicate that fermentation can slightly alter protein secondary structures, evidenced by a small increase in protein aggregates and a decrease in α-helix structures (Muñoz-Pina et al., 2024). The fermentation strains seem capable of disrupting the hydrogen bonds between the amino acids side-chains and the C=O residues, unfolding the α-helix structure (Muñoz-Pina et al., 2024). The higher protein content observed in the supernatants in this experiment might be due to similar mechanisms.

Similarly, lactic acid bacteria fermentation has been investigated in the *pacu* and *spotted sorubim* fish waste, and the fermentation media inoculated with *Lactobacillus brevis* NRRL-B 4527 containing 90 % *spotted sorubim* viscera and 10 % sugarcane molasses at 30.0 °C had the highest protein content at 44.6 ± 0.7 % (dry basis). Protein hydrolysates, which are proteins broken down into peptides of varying sizes through chemical or enzymatic processes (Yathisha et al., 2023), also

highlight the importance of understanding peptide profiles in fermented fish silage (Neira et al., 2024). Consequently, there is a need to explore the potential bioactive peptides for our sea bass viscera broth fermentation with L. *plantarum* and L. *casei*.

In conclusion, the lactic acid bacteria can significantly enhance extracting the protein content from the sea bass viscera into the fermentation supernatants. These findings provide new insights into how lactic acid bacteria can improve protein recovery from fish by-products, underscoring their potential for valorizing fish processing side streams.

3.3. Analysis of bioactive peptides

Six types of potential bioactive peptides, including antioxidants, anti-inflammatory, anticancer, antithrombotic, immunomodulating, and hypolipidemic compounds were identified in the fermentation solid matrices and supernatants, each with specific activity sequences. Table 1 presents the sequences associated with each bioactive property, while Fig. 5 displays the count of each type of bioactive peptide identified in the samples. As shown in Fig. 5 (A) and (B), fermentation of the sea bass viscera broth yielded 28 antioxidant sequences, 2 anti-inflammatory, 2 anticancer, 9 antithrombotic, 1 immunomodulatory, and 1 hypolipidemic activity sequence in the solid matrices and supernatants.

As for the antioxidant activity, the sequences include the AGPSIVH, HL, IKK, ADF, IY, EL, YVY, LHR, PHA, PHI, MY, GAH, KVI, PEL, IR, LK, TY, VKY, VW, YVGD, FIGP, ELLI, QYP, AGDDAPR, YNI, IDLGTTY, GPE, and YF for our solid matrices and supernatants. These sequences suggest that fermentation assisted with lactic acid bacteria can enhance the antioxidant properties of fish side streams. Notably, previous studies have demonstrated the beneficial effects of LAB-fermented abalone viscera in suppressing blood pressure elevation. For instance, in a previous study (Fujimura et al., 2021), abalone viscera fermented with L. *casei* 001 showed to have inhibitory effects on blood pressure elevation in vivo, suggesting that abalone viscera fermented with LAB may be a promising functional food material for blood pressure control. Similarly, *Lactiplantibacillus pentosus* (*L. pentosus*) SN001-fermented abalone viscera exhibited antihypertensive effects by inhibiting the angiotensin-converting enzyme, reducing blood pressure in vivo with a favorable safety profile (Yamanushi et al., 2022). Previous research has provided evidence that antioxidant peptides could be identified from solid waste from the fish sauce industry, and the results revealed that the fish sauce by-product (FSB) contained a high amount of proteins/peptides having antioxidant activity, and two peptide sequences (PQLLLLLL and LLLLLLL) were identified after fractionation using gel-filtration, the molecular weight cut-off filter, RP-HPLC and identification using LC-MS/MS (Choksawangkarn et al., 2018).

Besides, the anti-inflammatory activity includes the VPP and the PY, the anticancer activity consists of the YK and LKK, and the antithrombotic includes the GPRG, GPRGP, GPR, GP, PGP, PG, SSGE, DEE, and RGGD, the GLF of immunomodulating activity, and the EF of hypolipidemic activity. The antihypertensive effect of LAB in fermented fish has been summarized by a recent study (Liu, Berrada, Wang et al., 2024), that is based on the production of enzymes that reduce blood congestion and produce specific peptides that inhibit angiotensin-converting enzyme (ACE) or produce secondary metabolites inhibiting cholesterol synthesis.

Bioactive peptides from fermented milk by specific L. *plantarum* strains were assessed, with crude extracts showing higher activity than peptide fractions (<3 kDa and 3–10 kDa). Peptides <3 kDa generally outperformed or matched the 3–10 kDa fractions. Crude extracts and fractions from L. *plantarum* 55 demonstrated notable anti-inflammatory (723.68–1759.43 μg/mL diclofenac sodium equivalents), antihemolytic (36.65–74.45 % inhibition), and antioxidant activity (282.8–362.3 μmol Trolox equivalents) (Aguilar-Toalá et al., 2017). Similarly, fish hydrolysates/peptides exhibit a range of biological activities including antioxidant, lipid homeostasis modulation, anti-inflammatory, anticancer,

Table 1
The potential bioactivity sequences identified in the fermentation solid matrices and the supernatants with the sea bass viscera broth.

Activity	ID	Peptide Name	Sequence	Chem. mass
Antioxidative	9916	Antioxidative peptide	AGPSIVH	679.7630
	3305	Antioxidative peptide	LH	268.3114
	3317	Antioxidative peptide	HL	268.3114
	3319	Antioxidative peptide	HH	292.2932
	7862	peptide from prawn muscle (*Penaeus japonicus*)	IKK	387.5160
	7866	peptide from Okara protein	AY	252.2658
	7868	peptide from Okara protein	ADF	351.3536
	7872	peptide from soybean protein isolates: beta-conglycinin and glycinin	LY	294.3453
	7873	peptide from soybean protein isolates: beta-conglycinin and glycinin	IY	294.3453
	7886	peptide derived from egg white albumin	AH	226.2319
	7888	antioxidative peptide	EL	260.2861
	7911	synthetic peptide	WHH	478.5027
	7960	synthetic peptide	YVY	443.4917
	7984	synthetic peptide	HIH	405.4504
	7999	synthetic peptide	LHR	424.4966
	8016	synthetic peptide	LWR	473.5671
	8022	synthetic peptide	PHA	323.3468
	8023	synthetic peptide	PHD	367.3563
	8026	synthetic peptide	PHG	309.3203
	8027	synthetic peptide	PHI	365.4263
	8030	synthetic peptide	PHM	383.4659
	8090	peptide derived from sardine muscle	MY	312.3849
	8114	peptide derived from sardinelle by-products proteins (*Sardinella aurita*)	GGE	261.2313
	8115	peptide derived from sardinelle by-products proteins (*Sardinella aurita*)	GAH	283.2831
	8130	Antioxidative peptide	EAK	346.3784
	8133	peptide derived from dried bonito	KVI	358.4749
	8134	peptide derived from dried bonito	KD	261.2742
	8139	Antioxidative peptide	PEL	357.4010
	8150	peptide derived from bovine kappa-casein (30−32)	YVL	393.4760
	8215	Antioxidative peptide	IR	287.3576
	8216	Antioxidative peptide	LKP	356.4591
	8217	Antioxidative peptide	LK	259.3442
	8218	Antioxidative peptide	KP	243.3019
	8219	antioxidative peptide	TY	282.2917
	8224	antioxidative peptide	VY	280.3188

(*continued on next page*)

Table 1 (*continued*)

Activity	ID	Peptide Name	Sequence	Chem. mass
	8434	Antioxidant peptide from jellyfish (*Rhopilema esculentum*)	VKP	342.4326
	8445	Antioxidant peptide from marine bivalve (Mactra veneriformis)	TDY	397.3789
	8461	Antioxidant peptide from marine bivalve (Mactra veneriformis)	VW	303.3554
	8462	Antioxidant peptide from marine bivalve (Mactra veneriformis)	LW	317.3819
	8496	Antioxidant peptide from *ginkgo biloba*	YVGD	452.4572
	8973	Antioxidative peptide	GSGGL	389.4031
	8975	Antioxidative peptide	FIGP	432.5120
	8983	Antioxidative peptide	GAA	217.2218
	8984	Antioxidative peptide	ELLI	486.6005
	8987	Antioxidative peptide	GPP	269.2962
	9179	Antioxidative peptide	QYP	406.4319
	9438	Antioxidative peptide	VAPEEHPV	876.9505
	9443	Antioxidative peptide	AGDDAPR	700.6963
	9911	Antioxidative peptide	AGPSIVH	679.7630
	10,018	Antioxidative peptide	YNI	408.4477
	10,051	Antioxidative peptide	RY	337.3733
	10,157	Antioxidative peptide	IDLGTTY	781.8481
	10,226	Antioxidative peptide	LGF	335.3971
	10,378	Antioxidative peptide	GPE	301.2950
	10,470	Antioxidative peptide	YA	252.2658
	10,471	Antioxidative peptide	YF	328.3616
	10,539	Antioxidative	GC	178.2102
Anti-inflammatory	9538	Anti-inflammatory peptide	VPP	311.3757
	9856	Anti-inflammatory peptide	PY	278.3030
Anticancer	4017	Anticancer peptide	YK	309.3599
	5456	Anticancer peptide	LKK	387.5160
Antithrombotic	3039	Inhibitor of fibrinogen and thrombin coagulation	GPRPP	522.5963
	3040	Inhibitor of fibrinogen and thrombin coagulation	GPRG	385.4177
	3041	inhibitor of fibrinogen and thrombin coagulation	GPRGP	482.5326
	3042	inhibitor of fibrinogen and thrombin coagulation	GPRGPA	553.6103
	3043	inhibitor of fibrinogen and thrombin coagulation	GPRP	425.4814
	3044	Antithrombotic peptide	GPRGPP	579.6475
	3047	Antithrombotic peptide	GPR	328.3665
	3283	Antithrombotic peptide	GP	172.1813
	3284	Antithrombotic peptide	PGP	269.2962
	3285	Antithrombotic peptide	PG	172.1813
	3353	Antithrombotic peptide	SSGE	378.3343
	3354	Antithrombotic peptide	DEE	391.3298
	9660	Antithrombotic peptide	RGD	346.3388
Immunomodulating	9386	Immunostimulating peptide	GLF	335.3971
Hypolipidemic	9580	Hypolipidemic peptide	EF	294.3024

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.

neuroprotective, and antihypertensive effects promising nutraceutical ingredients for food applications (Gao et al., 2021). In addition to these health benefits, fish protein hydrolysates also possess functional properties like emulsification, foaming, and gelling, making them suitable as multipurpose ingredients (Gao et al., 2021). The process of creating fish protein hydrolysates typically involves breaking down fish waste into peptides containing 2 to 20 amino acids, which can be highlighted as valuable dietary supplements due to their diverse biological activities, including antioxidant, antimicrobial, immunomodulatory, and anticancer effects (Valério et al., 2023). This study provides valuable evidence of the multifunctional role of bioactive peptides derived from fermented, lipid-removed sea bass viscera through the action of lactic bacteria (L. *plantarum* and *L. casei*) strains. Consequently, fermented sea bass viscera could be considered for the development of bioactive products aimed at health promotion, reducing disease risk, or enhancing physiological functions.

3.4. ICP-MS analysis of minerals

Fish side streams can act as a source of proteins, lipids, and minerals such as calcium (Ca), phosphorus (P), potassium (K), sodium, magnesium, iron (Fe), zinc (Zn), manganese (Mg), and copper (Cu), and many other valuable components (Valério et al., 2023). The ICP-MS analysis of the mineral content in Fig. 6 was conducted after full fermentation with *Lactobacillus plantarum* and *Lactobacillus casei*.

For magnesium (Mg), concentrations were significantly higher in the fermentation supernatants compared to the solid matrices, with the values measured in fermentation solid matrices (0.3 mg/g in the viscera control, 0.2 mg/g in L. *plantarum*, and 0.2 mg/g in L. *casei*)) and the supernatants (2.9 mg/g in viscera control, 4.2 mg/g in L. *plantarum*, and 4.2 mg/g in *L. casei*). Moreover, the two lactic acid bacteria (L. *plantarum* and *L. casei*) facilitated a more efficient transfer of Mg from the sea bass viscera to the fermentation supernatants compared to the control, with L. *plantarum* showing a particular notable enhancement.

For phosphorus (P), the concentrations in the solid matrices were 4.1 mg/g for both the viscera control and L. *plantarum*, and 3.4 mg/g for L. *casei*. In the supernatants, the concentrations were 16.8 mg/g in viscera control, 19.9 mg/g in *L. plantarum*, and 19.3 mg/g in *L. casei*. A similar trend was observed for P, with most of the minerals found in the

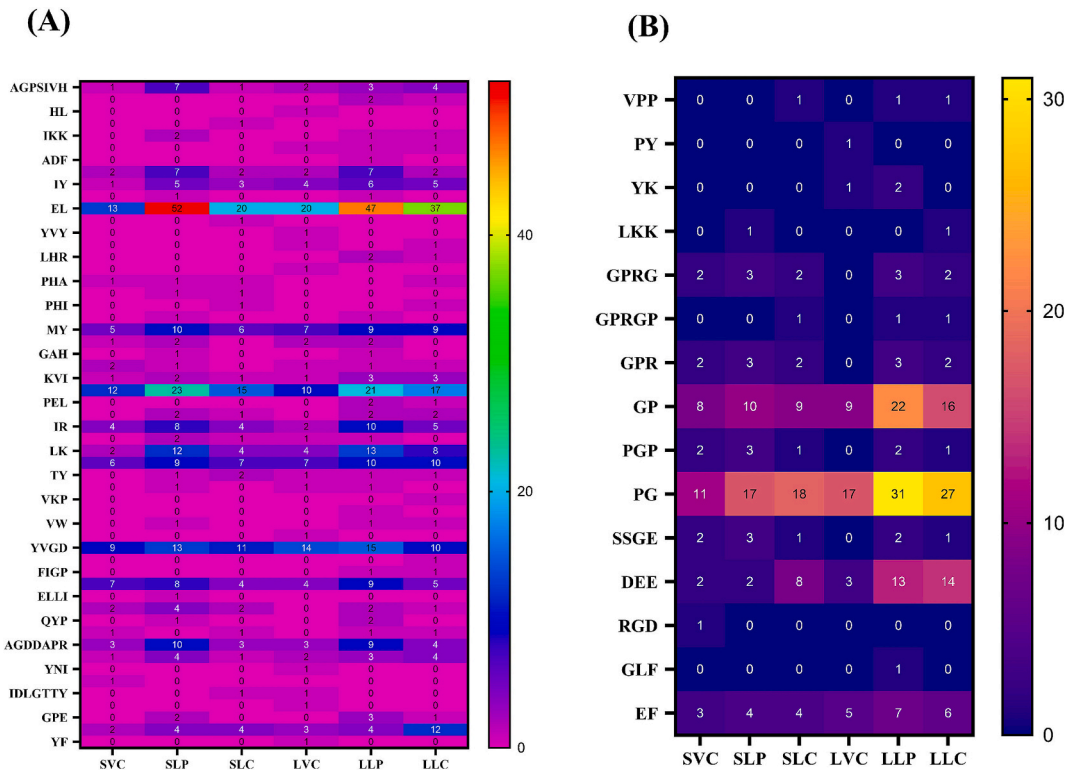

Fig. 5. Antioxidant activity (A) and anti-inflammatory, anticancer, antithrombotic, immunomodulating, and hypolipidemic activity (B) sequences for fermented supernatants and solid matrices object of the study.

fermentation supernatants. The highest concentration, 19.9 mg/g, was observed in the supernatants of L. *plantarum*.

For potassium (K) levels, the solid matrices showed concentrations of 1.9 mg/g in viscera control, 1.4 mg/g in *L. plantarum*, and1.3 mg/g in *L. casei*, while the supernatants contained 37.6 mg/g in viscera control, 45.2 mg/g in *L. plantarum*, and 43.9 mg/g in *L. casei*. The highest K concentration (45.2 mg/g) was found in the supernatants of *L. plantarum*.

For calcium (Ca), the concentrations in the solid matrices were 0.5 mg/g in viscera control, 0.4 mg/g in L. *plantarum,* and 0.4 mg/g in L. *casei*. The supernatants contained 2.6 mg/g in viscera control, 4.5 mg/g in *L. plantarum* and 4.8 mg/g in *L. casei*. The fermentation supernatants showed significantly higher Ca content than the solid matrices, with the highest value of 4.8 mg/g observed in the supernatants of L. *casei*.

For iron (Fe), the concentrations were measured in both the fermentation solid matrices (209.0 mg/Kg in viscera control, 220.0 mg/Kg in L. *plantarum*, and 183.1 mg/Kg in L. *casei*) and the supernatants (58.9 mg/Kg in viscera control, 51.7 mg/Kg in *L. plantarum*, and 54.5 mg/Kg in *L. casei*). The majority of Fe remained in the solid matrices after fermentation, with the highest value observed in the L. *plantarum* solid matrix at 220.0 mg/kg.

For copper (Cu), the concentrations in the solid matrices were 119.6 mg/Kg in viscera control, 121.0 mg/Kg in *L. plantarum* and 120.0 mg/Kg in *L. casei*. In the supernatants, the Cu content was 97.1 mg/Kg in the viscera control, 61.0 mg/Kg in *L. plantarum*, and 60.5 mg/Kg in *L. casei*. Most of the Cu remained in the solid matrices, with the highest concentration (121.0 mg/kg) found in the L. *plantarum* matrix.

As for the minerals of Zn, the solid matrices displayed concentrations of 117.0 mg/Kg in viscera control, 112.0 mg/g in *L. plantarum*, and 112.0 mg/g in *L. casei*. In the supernatants, the Zn content was 443.4 mg/Kg in viscera control, 100.8 mg/g in *L. plantarum*, and 106.3 mg/g in *L. casei*. The highest concentration (117.0 mg/kg) was found in the solid matrix of the viscera control.

For selenium (Se), with two significant function effects: in the removal of H_2O_2 and hydroperoxides; and synthesis of the active form of thyroid hormone (Lall, 2022). The concentrations for Se were similar in the solid matrices, with 3.6 mg/Kg in viscera control, 3.5 mg/g in *L. plantarum*, and 3.5 mg/g in *L. casei*. In the supernatants, the Se content was 2.1 mg/Kg in viscera control, 2.2 mg/g in *L. plantarum*, and 2.5 mg/g in *L. casei*. While the solid matrices showed no significant differences, the supernatants exhibited significant variations ($P < 0.05$), with L. *casei* yielding the highest Se content.

Minerals like calcium, phosphorus, magnesium, sodium, potassium, and chloride play vital roles in fish growth, development, and health (Lall, 2022). It is well-believed that the six macro elements which are essentially established by biochemical mechanisms (Lall, 2022), calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), and potassium (K), and chloride (Cl), are required in gram quantities. Utilizing the pulsed electric field (PEF) to recover the bioactive compounds from the sea bass side streams (head, skin, viscera, and muscle) with 100 % water or 50 % ethanol has also been investigated in both the solid matric and the liquid extracts, and this results large quantity of the minerals remain in the sea bass side streams and the PEF cooperated with different extraction solvent can promote the mineral recovery (Liu, Berrada, Wang et al., 2024). Moreover, the SFE prior to PEF to improve high-added-value compounds' recovery from salmon side streams (head, backbone, and viscera) illustrates that ICP-MS analysis revealed PEF helps solid matrices retain more minerals (Liu et al., 2025). The same trend can be seen in the Fe, Cu, Zn, and Se, and the opposite phenomenon can be observed in Mg, P, K, and Ca in Fig. 6. There is limited research on fermentation as a method for mineral recovery, while some mineral extraction from fish side streams has been explored. Our findings demonstrate that fermentation can effectively recover high quantities of minerals from fish side streams, indicating its potential as a viable approach to utilizing fish side streams.

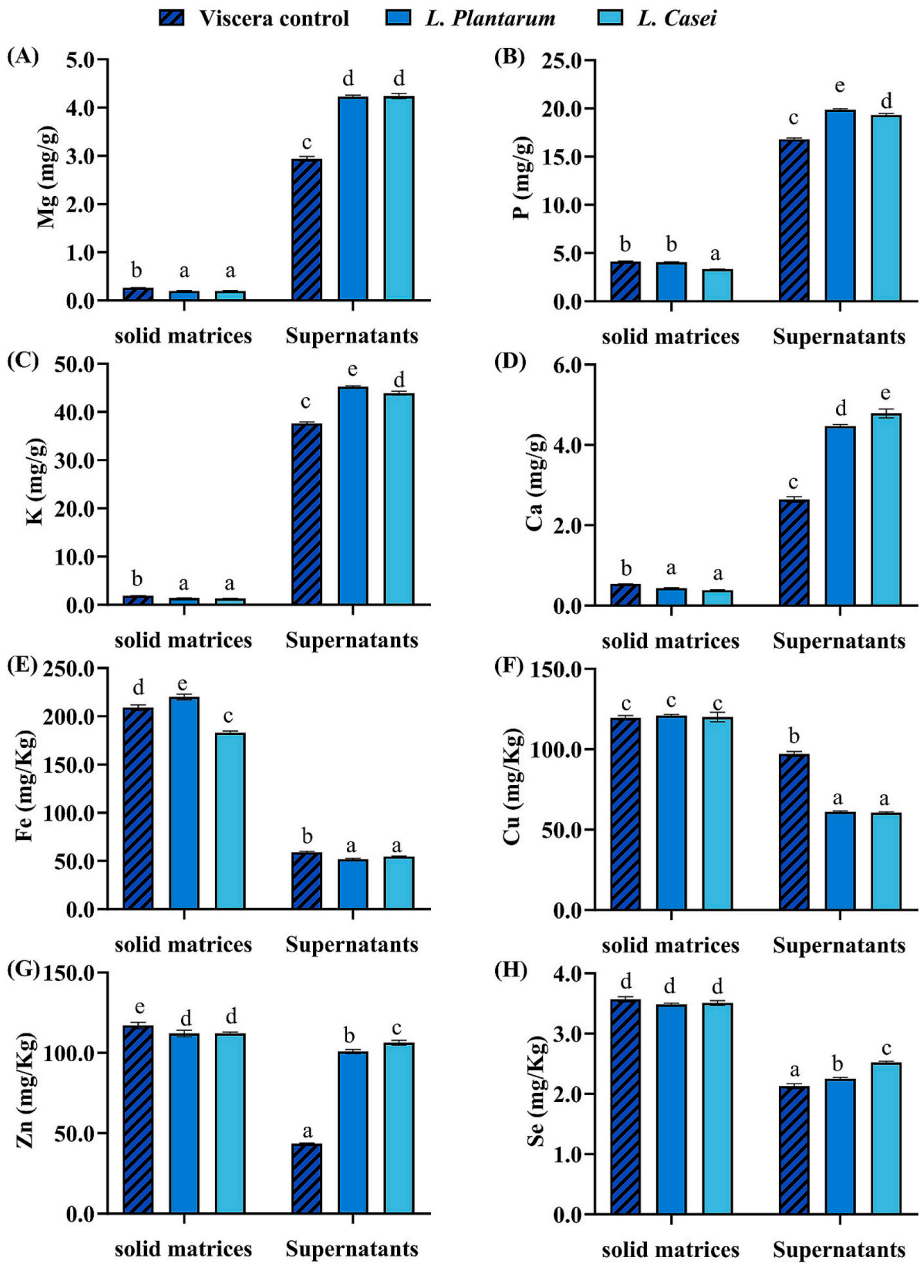

Fig. 6. Mineral profiles of solid matrices and supernatants obtained after the fermentation process with the sea bass viscera broth.

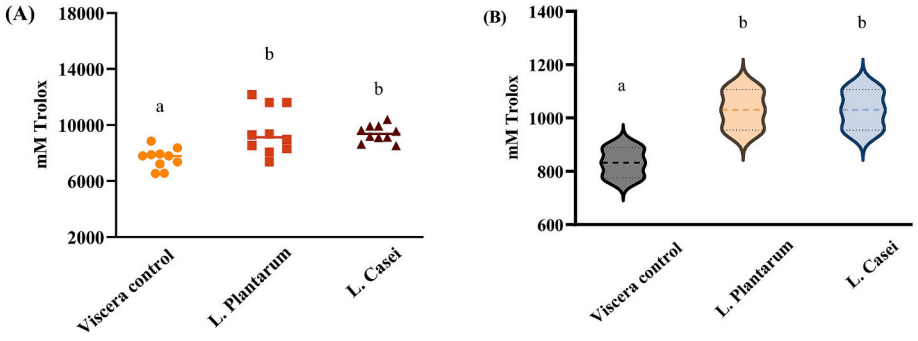

Fig. 7. The antioxidant capacity in ORAC (A) and the TEAC (B) of the fermentation solid matrices and supernatants after the fermentation process with the sea bass viscera broth.

3.5. Antioxidant capacity analysis

The ORAC was carried out in research to assess the antioxidant compounds from the sea bass viscera broth fermentation supernatants to scavenge peroxyl radicals, and the result was presented in Fig. 7(A). The ORAC values were for viscera control (7629.0 mM Trolox), *L. plantarum* (9521.8 mM Trolox), and L. *casei* (9400.0 mM Trolox). The sea bass viscera broth vaccinated with L. *plantarum* and *L. casei* displays a significant capacity to scavenge peroxyl radicals compared with the control. This improvement in antioxidative capacity likely results from the release of antioxidant compounds facilitated by L. *plantarum* and *L. casei* during fermentation.

The $ABTS^{+}$ scavenging ability results are presented in Fig. 7(B), showing a similar trend. Both L. *plantarum* and *L. casei* exhibited significantly higher $ABTS^{+}$ scavenging capacities compared to the viscera control, indicating that fermentation with these lactic acid bacteria boosts antioxidant activity.

Fish sauce by-product (FSB) refers to solid waste from the fish sauce industry (Choksawangkarn et al., 2018), the antioxidant activity of the FSB extract was determined using a 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay and the two most effective fractions had half-maximal inhibition values of 0.57 ± 0.05 mg/mL and 1.25 ± 0.16 mg/mL. Similarity, in vitro digestibility and angiotensin-converting enzyme (ACE) inhibitory activity of solid-state fermented fava beans (*Vicia faba* L.) with *Pleurotus ostreatus* strain, the antioxidative measured by ABTS which increased from the 3.13 to 5.09 mg TE/g DB due to the fungal fermentation (Muñoz-Pina et al., 2024).

4. Conclusions

In conclusion, this study utilized lipid-free sea bass viscera as a fermentation medium for L. *plantarum* and L. *casei*. Both LAB strains significantly increased ($P < 0.05$) the protein content in the supernatant for both L. *plantarum* and *L. casei* compared with viscera control samples. The fermentation process of the sea bass viscera broth yielded 28 antioxidant sequences, 2 anti-inflammatory, 2 anticancer, 9 antithrombotic, 1 immunomodulatory, and 1 hypolipidemic activity sequence in the fermentation solid matrices and supernatants. Especially, the EL (glutamic acid-phenylalanine) and KD (lysine-aspartic acid) for the antioxidant sequence, and the GP (glycine-proline) and the PG (proline-glycine) for the antithrombotic sequences are presented as the predominant bioactivity peptides sequences. The influence on protein extractability in supernatants and the six types of potential bioactive peptides was confirmed in this study, indicating the correlation between the potential digestion of protein and the potential development of bioactive peptides. Moreover, higher concentrations of Mg, P, K, and Ca were displayed in the fermentation supernatants, in contrast, more contents of the Fe, Cu, Zn, and Se were presented in the fermentation solid matrices after the fermentation process. Different trends in mineral extractability were found for the minerals in the solid matrices and the supernatants with the fermentation of L. *plantarum* and *L. casei*, indicating that the lactic acid fermentation can modify bioaccessibility for minerals, although further research is needed to confirm it. Besides, the antioxidant assays demonstrated that both LAB strains effectively enhanced the antioxidant properties of the fermented supernatants. Results of protein contents, bioactivity peptides, and minerals confirmed the fermented sea bass viscera's suitability for consumption, potential health application, and potential modification in protein digestibility and mineral bioaccessibility, which should be further studied in future works. In addition, the fermentation performed as the novel food processing poses a huge potential to recover high-added-value compounds from lipid-free sea bass side streams, such as more types of bioactive peptides and higher antioxidant capacity. Hence, fermentation may be applied to recover the high-added-value components from the marine resources, such as microalgae and macroalgae, not only focusing on the fish side streams.

CRediT authorship contribution statement

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

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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