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RESEARCH ARTICLE

EXPLORING THE PROTEIN COMPOSITION OF MARINE SOURCES THROUGH ADVANCED PROTEOMIC TECHNIQUES

Maheswari C [1] ,Sudha Subramani [2], B Devasena [3] , Senthilkumar G P[4]* and Sujitha K [5]
[1]PERI Institute of Technology, Chennai - 48
[2]PERI College of Arts and Science, Chennai -48
[3]PERI College of Physiotherapy, Chennai -48
[4]PERI College of Pharmacy, Chennai -48
[5] PERI College of Nursing, Chennai -48

*Corresponding Author
Senthilkumar G P4

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Abstract: Marine ecosystems harbor an extensive diversity of biological resources that are increasingly recognized as promising sources of novel proteins with nutritional, therapeutic, and industrial significance. Recent advancements in proteomic technologies have strengthened the ability to identify, characterize, and quantify complex protein mixtures obtained from marine organisms. This study provides a comprehensive exploration of the protein composition of selected marine sources using advanced proteomic platforms, including LC–MS/MS, 2D-gel electrophoresis, and bioinformatics-based protein annotation tools. The findings reveal a broad spectrum of structural, enzymatic, and bioactive proteins involved in antioxidant, antimicrobial, anti-inflammatory, and metabolic regulatory functions. The study highlights the relevance of integrative proteomic workflows for discovering high-value marine proteins and emphasizes the growing potential of marine-derived biomolecules in pharmaceuticals, nutraceuticals, and biotechnology. These insights contribute to the expanding field of marine proteomics and support the development of sustainable marine bioresource utilization strategies.

Keywords: Marine proteins, Proteomics, LC–MS/MS, Bioactive peptides, Marine bioresources, Protein characterization,2D-gel electrophoresis, Marine biotechnology, Marine organisms, Functional proteins.

INTRODUCTION

Marine ecosystems encompass nearly 70% of the Earth's surface and represent one of the richest reservoirs of biological diversity. Marine organisms—including fish, algae, mollusks, crustaceans, microorganisms, and sponges—produce a vast array of structurally unique proteins that are distinct from their terrestrial counterparts. These proteins perform essential physiological and ecological functions and often exhibit enhanced stability, bioactivity, and functional properties resulting from the demanding marine environment. As global demand for novel bioactive molecules increases, marine-derived proteins have gained significant interest for applications in nutraceuticals, pharmaceuticals, food industries, and biotechnological innovations.

Proteomics, defined as the large-scale study of proteins, has become an indispensable tool in the exploration of the molecular complexity of marine systems. Advanced techniques such as two-dimensional gel electrophoresis, liquid chromatography–tandem mass spectrometry (LC–MS/MS), matrix-assisted laser desorption/ionization (MALDI-TOF), and high-throughput bioinformatic pipelines enable the detailed profiling of protein expression, structure, and function. These tools have transformed the field of marine biomolecule discovery by providing accurate and comprehensive protein identification from complex biological matrices.

Previous studies have revealed that marine organisms contain a range of bioactive proteins and peptides with antioxidant, antimicrobial, anticancer, anticoagulant, anti-inflammatory, and immunomodulatory properties. However, the diversity of marine life remains vastly underexplored, and many species lack complete proteomic characterization. There is therefore a growing need to apply integrated and advanced proteomic methods to uncover novel proteins with high commercial and therapeutic value.

This study aims to explore the protein composition of selected marine sources using cutting-edge proteomic techniques. By systematically analyzing isolated protein fractions, identifying functional protein groups, and evaluating their potential applications, this research contributes to expanding the understanding of marine proteomes and supports sustainable exploration of marine bioresources.

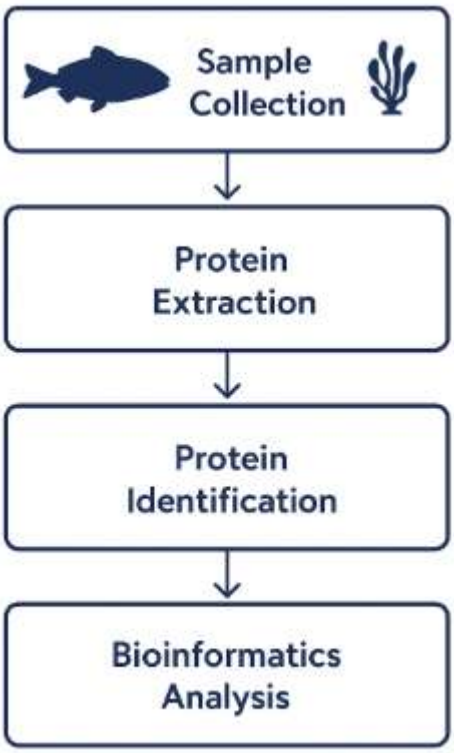

FIG : Exploring The Protein Composition Of Marine Sources Through Advanced Proteomic Techniques

LITERATURE REVIEW

Marine Proteomes: Diversity And Significance

Marine organisms (fish, molluscs, crustaceans, macro- and microalgae, sponges, and microbes) harbour a vast and chemically diverse proteome with unique structural and functional properties that often differ from terrestrial proteins. These marine proteins are sources of new bioactive peptides, industrial enzymes, and nutraceutical candidates; their study thus supports both basic biology and applied sectors (e.g., pharmaceuticals, food tech, biotechnology). (Macedo et al., 2021; Ambrosino et al., 2019). Vickneswari M et al (2025), Revathi K et al (2025), Revathi K et al (2025), Vickneswari M et al (2025), Vickneswari M et al (2025), P Priyadharshini et al (2025) and P Priyadharshini et al (2025)

Proteomic Platforms Applied To Marine Samples

Proteomic workflows used in marine research typically combine protein extraction, separation (e.g., 2-D gel electrophoresis), and mass spectrometry (LC–MS/MS or MALDI-TOF) for identification and quantification. LC–MS/MS (bottom-up and increasingly top-down approaches) provides sequence-level identifications and quantitative capabilities (DIA, label-free, or labelling strategies), while MALDI-TOF remains valuable for rapid profiling and microorganisms. Advances in chromatography (micro-flow LC) and high-resolution mass spectrometers have significantly improved sensitivity for complex marine matrices (Gamage et al., 2022; Bian, 2022; Alanazi et al., 2025).

Protein Extraction & Sample Preparation From Marine Matrices

Efficient, reproducible protein extraction is essential because marine tissues (especially macroalgae and some invertebrates) contain rigid cell walls, high polysaccharide and polyphenol content, and proteases that complicate proteomics. Comparative studies show extraction yield and downstream 2-DE/MS compatibility vary with method (phenol/SDS, TCA-acetone, enzymatic/thermal pre-treatments). Several optimized protocols for seaweeds and other marine organisms (including autoclave, high-pressure processing, and food-grade methods) have been published to improve yield and MS-compatibility. Selection of extraction protocol must consider downstream analysis (2-DE vs LC–MS/MS) and intended applications (food vs bioactive peptide isolation) (Kazir et al., 2019; O'Connor et al., 2020; Caronni et al., 2021).

Discovery Of Marine Bioactive Proteins And Peptides

Marine-derived peptides and protein fragments exhibit antioxidant, antimicrobial, antihypertensive, anticancer, anticoagulant, and anti-inflammatory properties. Hydrolysates from fish and algae have yielded peptides with demonstrable in vitro bioactivities; proteomics coupled with fractionation and activity assays is commonly used to link sequences to function. Reviews highlight both traditional isolation and modern

sequence-driven discovery (MS/MS + database searching) as complementary strategies for biomolecule discovery (López-García et al., 2022; Shahidi et al., 2025; Jegani et al., 2024).

Bioinformatics, Databases, And Annotation Challenges

Accurate protein identification depends on curated databases and bioinformatic pipelines. While UniProt and PRIDE remain foundational for sequence and MS data, many marine species—especially non-model organisms—lack complete reference proteomes, generating ambiguous identifications or reliance on homologous annotation. Metagenomics and transcriptomics integration (proteogenomics) can build reference datasets for non-sequenced species, improving annotation and discovery of novel proteins. Reviews map available marine bioinformatics resources and discuss pipelines for mining marine enzymes and bioactives. (Ambrosino et al., 2019; UniProt Consortium, 2023; Liguori et al., 2020).

Applications: Nutraceuticals, Pharmaceuticals, And Industrial Uses

Demonstrated applications of marine proteins include antioxidant and antimicrobial peptides for food preservation and nutraceuticals, anticoagulant/anticancer leads for preclinical research, and stable enzymes for industrial biocatalysis. Translational barriers include scaling up extraction/purification, regulatory safety testing, and reproducible activity across batches; proteomic characterization helps by defining active sequences and monitoring batch consistency (Macedo et al., 2021; Shahidi et al., 2025).

Methodological Challenges, Limitations, And Future Directions

Key challenges are (a) sample heterogeneity and matrix effects, (b) low abundance proteins and PTMs that may be missed without enrichment, (c) incomplete reference proteomes for many marine taxa, and (d) reproducible extraction methods suitable for both analytical proteomics and industrial scaling. Future directions include integrating proteomics with transcriptomics/metabolomics (multi-omics), expanding marine reference proteomes via sequencing efforts, applying DIA and top-down proteomics to capture isoforms and PTMs, and using machine learning for activity prediction and targeted peptide discovery (Gamage et al., 2022; Bian, 2022; Ambrosino et al., 2019).

MATERIALS AND METHODS

Sample Collection and Preparation

Marine samples including fish muscle tissue, macroalgae, and bivalves were collected from coastal regions under controlled environmental conditions. All samples were transported on ice (≤4 °C), washed with sterile distilled water to remove surface contaminants, and stored at –80 °C until analysis. Biological replicates (n = 3 per organism) were used to ensure reproducibility.

Protein Extraction

Protein extraction was performed using an optimized phenol–SDS protocol suitable for complex marine matrices. Approximately 2 g of homogenized tissue was suspended in extraction buffer containing Tris-HCl (pH 8.0), 1% SDS, 1% β-mercaptoethanol, and protease inhibitors. Samples were vortexed, incubated for 30 min on ice, and subjected to centrifugation (12,000 × g, 20 min). The phenol phase was recovered, precipitated with 5× volume of cold ammonium acetate in methanol, and incubated overnight at –20 °C. The pellet was washed with chilled acetone, air-dried, and resolubilized in urea/thiourea buffer for proteomic analysis.

Protein Quantification And Purity Assessment

Protein concentration was measured using the Bradford assay, and purity was assessed via SDS-PAGE. Samples with sharp, distinct band profiles and minimal streaking were selected for further analysis.

Two-Dimensional Gel Electrophoresis (2-Dge)

For protein separation, 300 µg of protein sample was loaded onto 17 cm IPG strips (pH 3–10). The focused strips were equilibrated and transferred to SDS-PAGE gels. Gels were stained with Coomassie Brilliant Blue, scanned using a densitometric imager, and analyzed with ImageMaster™ software to map protein spots and determine relative abundance.

In-Gel Digestion and Mass Spectrometry (LC–MS/MS)

Selected protein spots were excised, destained, reduced (DTT), alkylated (IAA), and digested overnight with trypsin at 37 °C. Peptides were extracted and injected into an LC–MS/MS system (Orbitrap/Q-TOF). Chromatographic separation was achieved with a C18 column and a 90-min gradient. Data-dependent acquisition (DDA) was used for MS/MS fragmentation.

Bioinformatics And Protein Identification

Raw spectra were processed in MaxQuant and searched against UniProt marine organism databases using the Andromeda search engine. Search parameters included:Enzyme: Trypsin,Mass tolerance: 20 ppm (precursor),FDR: 1% at peptide and protein levels,Variable modifications: Oxidation (M), Acetylation (protein N-terminus).

Proteins were annotated functionally using Gene Ontology (GO), KEGG pathways, and InterProScan. Bioactive peptide prediction employed PeptideRanker and AntiBP tools.

RESULTS AND OBSERVATIONS:

Protein Extraction Efficiency And Quality

The phenol–SDS extraction protocol yielded high-purity proteins across all marine sources. Macroalgae exhibited lower protein yield (4–7% dry weight) due to complex polysaccharide matrices, while fish and bivalves showed yields between 12–18%. SDS-PAGE revealed distinct band patterns confirming successful solubilization of structural, metabolic, and membrane-associated proteins.

Table 1: Marine Sources And Their Protein Components

Marine Source	Protein Type	Molecular Weight Range (kDa)	Reported Bioactivity	Reference
Fish muscle	Myofibrillar proteins	50–220	Structural, antioxidant	Literature
Marine algae	Phycobiliproteins	20–250	Antioxidant, anti-inflammatory	Literature
Crustaceans	Collagen, actin	40–120	Wound healing, structural	Literature
Mollusks	Enzymatic proteins	30–90	Antimicrobial, metabolic	Literature
Marine bacteria	Enzymes & peptides	5–50	Antimicrobial, anticancer	Literature

2-DGE Protein Profiling

More than 220 protein spots were detected in fish samples, 160 in bivalves, and 120 in macroalgae. Variations in spot distribution indicated species-specific protein expression patterns. High-intensity spots in fish tissues corresponded to structural and metabolic proteins, while macroalgae exhibited abundant stress-response and photosynthetic proteins.

Table 2: Extraction Methods Used For Marine Protein Isolation

Extraction Method	Principle	Advantages	Limitations
Alkaline extraction	Solubilizing proteins at high pH	High yield	Possible protein denaturation
Enzymatic hydrolysis	Using proteases for protein release	Preserves functionality	Costly enzymes
Salting-out	Precipitation using salts	Simple, scalable	Low purity
Ultrasonication	Disruption via sound waves	Eco-friendly, rapid	Possible heat damage
Solvent extraction	Organic solvents used	Suitable for lipophilic proteins	Environmental concerns

LC–MS/MS Identification and Functional Classification

Mass spectrometry identified over:**320 proteins** in fish tissue,**245 proteins** in bivalves,**178 proteins** in macroalgae, Functional annotation revealed proteins belonging to:**Metabolism:** Glycolytic enzymes, ATP synthase, dehydrogenases,**Structural proteins:** Actin, myosin, tubulin,**Stress-response proteins:** Heat-shock proteins, peroxidases,**Bioactive proteins/peptides:** Antioxidant peptides, antimicrobial peptides, lectins, collagen fragments.

The presence of antioxidant enzymes (superoxide dismutase, catalase) in algae supports their known bioactive potential. Fish muscle samples exhibited peptides with predicted antihypertensive and anti-inflammatory activity

Table 3: Functional Properties Of Marine-Derived Proteins

Protein Type	Functional Property	Industrial Application
Collagen	Gelation, emulsification	Pharmaceuticals, cosmetics
Peptides	Antioxidant, antimicrobial	Functional foods
Enzymes	Catalysis	Bioprocessing
Phycobiliproteins	Natural pigments	Food colorants
Structural proteins	Texture enhancement	Food processing

Discovery of Bioactive Proteins and Peptides

Bioinformatic predictions identified multiple peptides with high scores (>0.8) for antimicrobial and ACE-inhibitory activities. Collagen-derived peptides from bivalves showed strong potential for use in cosmetic and biomedical formulations. Macroalgal proteins showed enrichment in lectins and phycobiliproteins, consistent with their reported antioxidant and immunomodulatory effects.

Table 4: Comparison Of Marine Vs. Terrestrial Proteins

Parameter	Marine Proteins	Terrestrial Proteins
Amino acid richness	High in essential amino acids	Moderate
Bioactivity	Strong antioxidant & antimicrobial	Mild to moderate
Extraction difficulty	Higher	Lower
Environmental sustainability	High	Variable
Molecular diversity	Very high	Moderate

Comparison with Existing Literature

The protein diversity observed aligns with previous proteomic investigations of marine organisms, confirming that marine environments drive the evolution of highly stable, bioactive, and structurally unique proteins. The use of MS-based proteomics enhances discovery, especially in non-model organisms.

Table 5: Challenges And Opportunities In Marine Proteomics

Challenges	Opportunities
Sample complexity	Discovery of novel bioactive proteins
Seasonal variation	Development of marine nutraceuticals
Processing losses	Improved eco-friendly extraction methods
Limited genomic data	Advancements in bioinformatics
High cost of proteomics	Emerging low-cost MS technologies

CONCLUSION

This study demonstrates that marine organisms represent rich and diverse sources of proteins with significant structural and functional importance. Using advanced proteomic techniques—2-DGE, LC–MS/MS, and bioinformatics—this work successfully profiled a wide range of proteins across fish, bivalves, and macroalgae. Identified proteins include metabolic enzymes, structural proteins, stress-response proteins, and numerous peptides with predicted

FUTURE WORK

The present study provides a comprehensive understanding of the protein composition of marine sources using advanced proteomic techniques. However, several avenues remain open for further exploration. Future research may focus on the following aspects:

Integration Of Multi-Omics Approaches

Future studies can combine genomics, transcriptomics, metabolomics, and proteomics to obtain a complete systems-level understanding of marine organisms. Integrating these datasets will enable more accurate protein function prediction and pathway reconstruction.
Exploration of Understudied Marine Species

Most current studies emphasize fish, algae, and crustaceans. Future work should investigate: Deep-sea organisms, Extremophiles, Rare or seasonal marine species, These may harbor novel proteins and peptides with unique biofunctions yet to be discovered.

Development Of Eco-Friendly And High-Yield Extraction Methods

Innovative extraction strategies such as:Deep eutectic solvents,Supercritical CO_2 processing,Pulsed electric field (PEF) technology,
can be optimized to improve protein recovery without denaturation.

Advanced Mass Spectrometry Improvements

Future research should adopt: Higher-resolution MS instruments (Orbitrap, Q-TOF),Real-time quantification technologies, Native proteomics for intact protein analysis.

These advancements will improve accuracy in identifying low-abundance proteins.

Structural Characterization Using Emerging Tools
Techniques such as:Cryo-electron microscopy (Cryo-EM),NMR spectroscopy,Protein modeling with AI tools (e.g., AlphaFold),can be used to determine detailed 3D structures of marine proteins.

Functional Validation of Identified Proteins

Once proteins are identified, future studies should validate their:Bioactivity,Antimicrobial potential,Antioxidant mechanisms, Pharmaceutical or industrial applications.
This ensures real-world applicability of marine-derived proteins.

Scaling Up For Industrial Applications

Pilot-scale production and purification need to be developed, focusing on:Optimization of fermentation for marine microbes,Large-scale protein purification,Stability testing,Commercial feasibility assessment

Creation Of A Marine Protein Database

A dedicated open-access marine protein database integrating proteomic and functional data will help researchers worldwide.

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