

VOLUME 11 ISSUE 1 2025

ISSN 2454 – 3055



**INTERNATIONAL
JOURNAL OF
ZOOLOGICAL
INVESTIGATIONS**

***Forum for Biological and
Environmental Sciences***

Published by Saran Publications, India



From Biomass to Biomolecules: A Review of Protein and Peptide Extraction Techniques from Biological Sources

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Received: 11th June, 2024; **Accepted:** 14th October, 2024; **Published online:** 13th January, 2025

<https://doi.org/10.33745/ijzi.2025.v11i01.004>

Abstract: The extraction of proteins and peptides from biomass sources plays a critical role in various fields, including biotechnology, pharmaceuticals, and food science. This review provides a comprehensive overview of the techniques used for extracting proteins and peptides from biological sources, ranging from traditional methods to advanced technologies. We discuss the principles behind each extraction technique, including solubilization, separation, and purification processes. Additionally, we explore the advantages and limitations of different extraction methods, considering factors such as yield, purity, and scalability. Furthermore, we highlight recent advancements and innovations in protein and peptide extraction, including emerging technologies and novel approaches. By exploring current knowledge and identifying areas for future research, this review aims to contribute to the development of efficient and sustainable extraction processes for biomolecules from biomass sources.

Keywords: Proteomics, Enzymes, Homogenization, Bioinformatics, Nutrition, Biomolecules

Citation: Narkhede Minal R., Nachan Yatin S. and Pande Sakshi D.: From biomass to biomolecules: A review of protein and peptide extraction techniques from biological sources. Intern. J. Zool. Invest. 11(1): 35-51, 2025.

<https://doi.org/10.33745/ijzi.2025.v11i01.004>



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Introduction

Biomass to Biomolecules explores the methods and techniques used to extract proteins and peptides from various biological sources, including plant, microbial, and animal origins. These essential biological entities play critical roles in various biological processes and unlock insights into cellular function. This combines cutting-edge technologies and traditional methodologies to uncover the secrets within biomass,

offering a profound understanding of the world of biomolecules. This comprehensive review bridging fundamental research and practical applications in biotechnology. It highlights principles like solubility, selectivity, and yield optimization, as well as emerging technologies and innovative approaches. The overview covers traditional methods like organic solvents and precipitation, as well as cutting-edge techniques

like chromatography and affinity-based isolation. The field evolves in response to technological advancements and scientific discoveries, providing a foundation for further exploration of specific extraction techniques. Efficient extraction techniques for proteins and peptides from biological sources hold paramount significance across a spectrum of scientific, industrial, and medical domains (Bilal and Iqbal, 2020). The effectiveness of these extraction methods directly influences the quality and quantity of isolated biomolecules, thereby impacting the outcomes of downstream analyses and applications. Several key aspects underscore the importance of developing and employing efficient extraction techniques in this context.

Biomedical Research Advancements

Efficient extraction techniques are foundational to biomedical research, enabling scientists to investigate the structure and function of proteins and peptides (Peluso and Chankvetadze, 2023). The success of experiments, such as structural elucidation, functional studies, and biomarker discovery, relies heavily on obtaining pure and intact biomolecules through effective extraction methods.

Diagnostic and Therapeutic Applications

In the realm of medicine, precise and reliable extraction of proteins and peptides is crucial for diagnostic tests and therapeutic interventions. Biomarkers extracted from biological samples can serve as indicators of disease states, facilitating early detection and monitoring of various medical conditions.

Biotechnological and Pharmaceutical Processes

Industries harness protein and peptide extraction for the production of biopharmaceuticals and other biotechnological applications. Efficient extraction techniques contribute to cost-effective and scalable manufacturing processes, ensuring the consistency and purity of the final products.

Environmental and Agricultural Applications

Proteins derived from biological sources play essential roles in various environmental and agricultural applications, including soil improvement, crop protection, and waste treatment. Extracting these biomolecules efficiently is vital for developing sustainable solutions in these fields.

Food and Nutrition Sciences

In the food industry, protein and peptide extraction is integral to the development of nutritional supplements and functional foods. Efficient extraction methods are essential for preserving the nutritional value of proteins and peptides in food products.

Resource Optimization

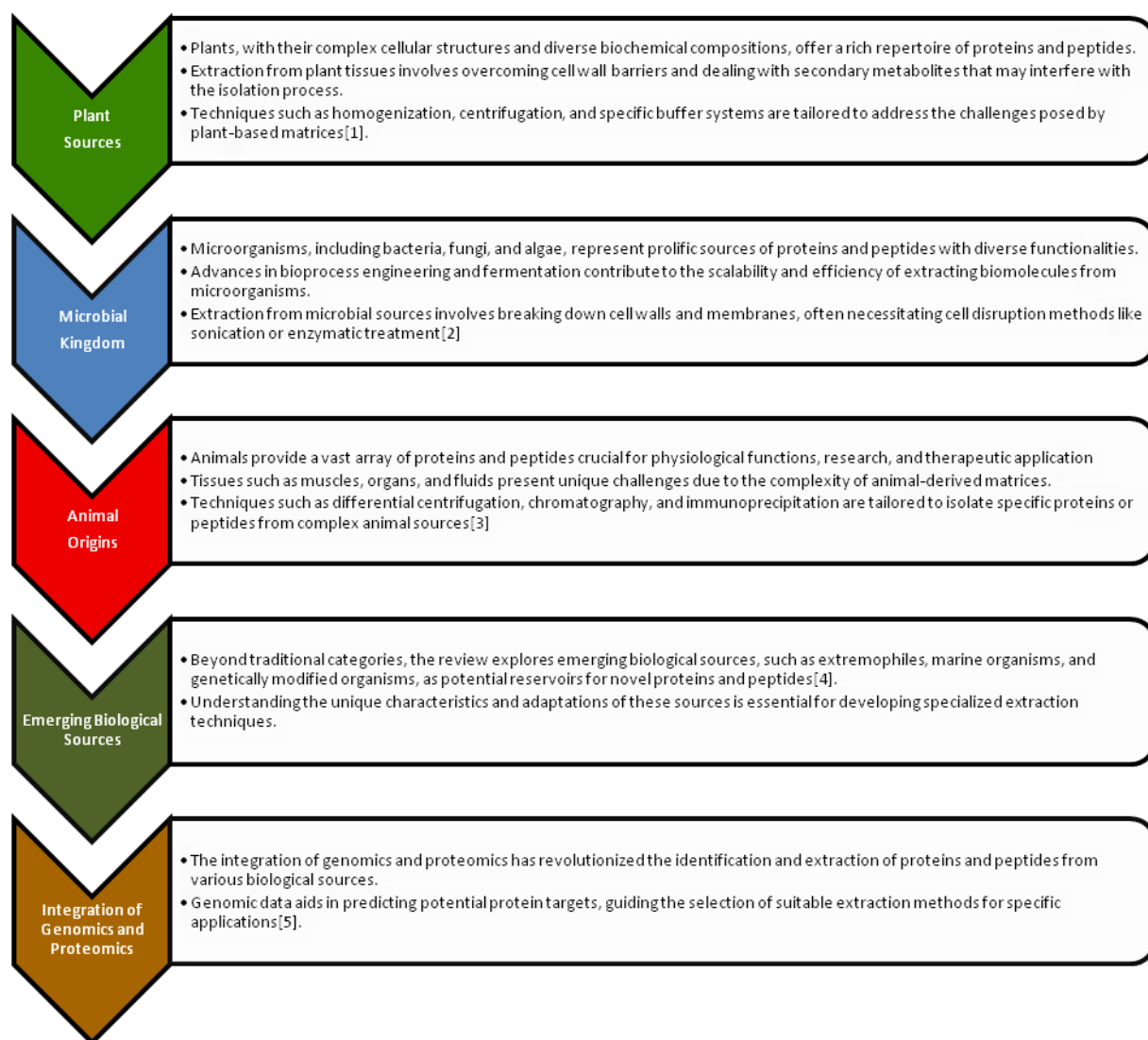
Efficient extraction techniques contribute to resource optimization by minimizing the consumption of solvents, reagents, and energy. This aligns with the principles of green chemistry and sustainable practices, addressing environmental concerns associated with laboratory and industrial processes.

Technological Advancements

Ongoing advancements in extraction technologies, such as high-throughput methods and automation, enhance the speed and reproducibility of the extraction process. This, in turn, accelerates the pace of scientific discovery and application development.

Biological Sources of Proteins and Peptides

The realm of protein and peptide extraction is intricately linked to the diversity of biological sources from which these essential biomolecules are derived. This section of the review focuses on categorizing and exploring the various biological origins, spanning the plant, microbial, and animal kingdoms, that serve as reservoirs for proteins and peptides. Each source brings forth unique challenges and opportunities, shaping the extraction techniques employed in this multifaceted field.



(A) *Plant-based sources:*

Plants represent a rich and diverse source of proteins and peptides, playing pivotal roles in ecological systems, human nutrition, and various industrial applications. Extracting these biomolecules from plant-based sources presents unique challenges owing to the complex cellular structures, diverse secondary metabolites, and variable concentrations across different tissues (Hu, 2003). This section delves into the intricacies of protein and peptide extraction from plant materials, elucidating key methodologies and considerations.

Plant cells have rigid cell walls, making them difficult to extract proteins and peptides. Homogenization techniques are used to break

down these walls, balancing efficiency with biomolecular integrity. Centrifugation and clarification are used to separate cellular debris and isolate proteins and peptides. Specific buffer systems are used to solubilize proteins and peptides, while reducing interference from secondary metabolites (Table 1). Differential extraction methods target specific classes of proteins, such as membrane proteins or soluble proteins. Specialized extraction techniques, like enzyme-assisted or supercritical fluid extraction, are used to isolate specific biomolecules. Challenges in plant-based extraction include the presence of polyphenols, protease inhibitors, and other secondary metabolites. Future developments may involve integrating omics data and

Table 1: Examples and methods of extraction of proteins and peptides from Plant sources

Sources	Considerations	Examples	Details	References
Plant Sources	Tissue Complexity and Cellular Barriers	a) Wheat Bran	Rich in fibre and outer layers, requiring efficient extraction methods to access proteins within.	Janssen <i>et al.</i> (2023)
		b) Corn Husks	Cellulosic barriers necessitate homogenization for protein extraction.	Cheran <i>et al.</i> (2024)
		c) Coconut Coir	Fibrous structure presents challenges in breaking down cell walls for protein release.	Mazumder and Zhang (2023)
		d) Sugar Beet Pulp	Contains complex cell wall components, requiring specialized.	Mondal and Katiyar (2024)
		e) Oat Hulls	Rigid structures demand mechanical disruption for effective protein extraction.	Meyllianawaty <i>et al.</i> (2024)
	Homogenisation Techniques	a) Soyabeans	Grinding or blending breaks down cell walls, facilitating protein release.	Umaru <i>et al.</i> (2024)
		b) Almonds	Mechanical disruption is essential for accessing proteins in almond kernels.	Shi <i>et al.</i> (2023)
		c) Pumpkin seeds	Homogenization aids in breaking down cell structures for protein extraction.	Ramondo <i>et al.</i> (2024)
		d) Sunflower seeds	Grinding is employed to release proteins embedded in seeds.	Arrutia <i>et al.</i> (2020)
		e) Quinoa	Blending helps disrupt the outer coating, allowing for protein extraction.	Mahdavi Yekta <i>et al.</i> (2020)
	Centrifugation and Clarification	a) Spinach Leaves	Centrifugation separates cellular debris from protein-containing supernatant.	Valente <i>et al.</i> (2022)
		b) Broccoli Florets	Clarification steps are crucial for obtaining a pure protein extract.	Thery <i>et al.</i> (2020)
		c) Cabbage	Centrifugation is employed to isolate proteins after homogenization.	Ali Al Jabr <i>et al.</i> (2020)
		d) Kale	Clarification enhances the purity of proteins extracted from kale.	Prade <i>et al.</i> (2021)

		e) Lettuce	Centrifugation aids in separating cellular components for protein isolation.	Trigo <i>et al.</i> (2021)
	Buffer Systems for Protein Solubilization	a) Barley seeds	Detergent-containing buffers solubilize proteins while mitigating interference.	Jaeger <i>et al.</i> (2021)
		b) Rye Flour	Specific buffer formulations enhance the solubility of proteins in rye.	Deleu <i>et al.</i> (2020)
		c) Chickpeas	Buffer systems are tailored to solubilize proteins while preserving their integrity.	Kumar <i>et al.</i> (2022)
		d) Lentils	Detergents and reducing agents are incorporated for optimal protein solubilization.	Islam <i>et al.</i> (2023)
		e) Kidney Beans	Buffer composition is adjusted to enhance the solubility and stability of extracted proteins.	Higa <i>et al.</i> (2024)
	Differential Extraction	a) <i>Arabidopsis thaliana</i> (Thale's cress)	Differentially extracts proteins based on solubility for diverse studies.	Laihonen <i>et al.</i> (2024)
		b) Maize	Sequential extraction isolates different classes of proteins for analysis.	Solomon <i>et al.</i> (2023)
		c) Sorghum	Differential extraction methods target specific protein fractions for research purposes.	Kamal <i>et al.</i> (2021)
		d) Sweet Potatoes	Different solubilization steps are employed for varied protein classes.	Ghoshal and Kaur (2023)
		e) Tomatoes	Sequential extraction allows for the isolation of proteins.	Yu <i>et al.</i> (2023)

advanced extraction technologies for precise targeting of proteins and peptides from specific plant sources.

(B) *Animal-derived sources:*

Animal-derived sources provide a vast array of proteins and peptides crucial for various applications in biomedical research, food production, and pharmaceuticals (Table 2). This section explores the intricacies of extracting proteins and peptides from animal-based matrices, highlighting specific methodologies and considerations.

Understanding the nuances of protein and peptide extraction from diverse animal sources is vital for researchers and industries aiming to harness the functional and therapeutic potential of these biomolecules. Each source presents unique challenges, necessitating tailored extraction strategies.

(C) *Microbial sources:*

Microorganisms as prolific sources: Microorganisms, including bacteria, fungi, and algae, are abundant sources of proteins and peptides with industrial applications (Table 3). Bacteria like *E.*

Table 2: Examples and methods of extraction of proteins and peptides for Animal sources

Sources	Considerations	Examples	Details	References
Animal Sources	Tissue Diversity and Complexity	a) Chicken Muscle Tissue	Extraction from muscle tissue involves breaking down connective tissues and myofibrillar structures.	Wang <i>et al.</i> (2024)
		b) Liver (Pig/Cow)	Liver tissue extraction requires addressing the presence of enzymes and cellular membranes.	Llauger <i>et al.</i> (2024)
		c) Fish Scales	Extracting proteins from scales involves dealing with unique structural components and the potential presence of bioactive peptides.	Rajabimashha di <i>et al.</i> (2023)
		d) Egg Whites	Extraction involves separating proteins like ovalbumin, conalbumin, and lysozyme from the complex matrix of egg white.	Liu <i>et al.</i> (2020)
		e) Bovine Blood serum	Extraction involves careful handling to obtain serum proteins, including albumins and globulins.	Liu <i>et al.</i> (2020)
	Homogenization & Tissue Disruption.	a) Brain Tissue (Rat or Mouse)	Homogenization is essential for breaking down complex neural tissues and obtaining brain-specific protein.	Xu <i>et al.</i> (2023)
		b) Fish Eyes	Mechanical disruption is employed to release proteins from the intricate eye structures.	Rana <i>et al.</i> (2023)
		c) Chicken Cartilage	Homogenization is necessary to access proteins embedded in the tough extracellular matrix.	Lu <i>et al.</i> (2023)
		d) Pork Skin	Mechanical processing is used to break down the collagen-rich structure for protein extraction.	Dewi <i>et al.</i> (2023)
		e) Shellfish Exoskeleton	Mechanical methods are applied to disrupt exoskeletons and release proteins from crustaceans.	Rebouças <i>et al.</i> (2023)
	Centrifugation Fractionation and	a) Bovine Milk Centrifugation.	Separation of proteins like casein and whey from the liquid component by using centrifuge machines.	Haller (2023)
		b) Chicken Egg Yolk	Centrifugation aids in isolating proteins from egg yolk, including lipovitellin and positing.	Mourad <i>et al.</i> (2023)

		c) Bone Marrow (Cow)	Centrifugation and fractionation are used to obtain proteins from the complex bone marrow matrix.	Huang <i>et al.</i> (2024)
		d) Blood Plasma (Human)	Centrifugation separates plasma proteins, including fibrinogen and immunoglobulins.	Si <i>et al.</i> (2024)
		e) Pig Intestinal Mucosa.	Fractionation methods help isolate proteins from the mucosal layer of the intestine.	Mamone and Picariello (2023)
	• Enzymatic & Chemical Digestion.	a) Bee Venom	Enzymatic digestion is employed to isolate peptides from complex venom compositions.	von Reumont <i>et al.</i> (2022)
		b) Stomach Digesta (Ruminant)	Chemical digestion is used to break down food particles and extract proteins from the stomach.	van Rensburg (2024)
		c) Pig Pancreas	Enzymatic digestion liberates proteins from pancreatic tissues.	Lu <i>et al.</i> (2021)
		d) Rat kidneys	Chemical digestion aids in extracting proteins from the kidney's complex cellular structure.	McCabe <i>et al.</i> (2023)
		e) Insect Haemolymph	Enzymatic methods are applied for extracting peptides from insect circulatory fluids.	Gianazza <i>et al.</i> (2021)
	• Challenges and Ethical Considerations	a) Frog Skin Secretions.	Ethical considerations arise in extracting bioactive peptides from amphibian secretions.	Indriani <i>et al.</i> (2023)
		b) Ostrich Eggs	Challenges include the limited availability of ostrich eggs and the need for specialized extraction methods.	Kheiralipour <i>et al.</i> (2024)
		c) Snake Venom	Ethical considerations and safety precautions are crucial in handling and extracting proteins from snake venom.	Halm (2024)
		d) Shark Cartilage	Extraction challenges involve the dense and calcified nature of shark cartilage.	Ermakova <i>et al.</i> (2024)
		e) Whale Blubber	Extraction poses challenges due to the unique lipid-rich composition of blubber.	Escher <i>et al.</i> (2023)

Table 3: Examples and methods of extraction of proteins and peptides for Microbial sources

Microbial Sources	Cell Disruption Methods	a) Sonication	High-frequency ultrasound waves generate cavitation forces that disrupt microbial cells, allowing the extraction of intracellular components. For example, sonication is often used to extract proteins from <i>E. coli</i> cells for various research or biotechnological applications	Jawed (2023)
		b) Enzymatic Treatment	Cell wall-degrading enzymes like lysozyme or zymolase can be used to break down microbial cell walls selectively. These enzymes are commonly used for the extraction of proteins and peptides from yeast or fungal cells.	Steinbrunh <i>et al.</i> (2023)

coli and *Bacillus subtilis* produce enzymes, antimicrobial agents, and bioactive peptides. Fungi like *Aspergillus* and *Penicillium* produce secondary metabolites with pharmaceutical, agricultural, and industrial potential. Algae like *Chlorella* and *Spirulina* are also explored as sources of proteins and peptides. Advances in bioprocess engineering and fermentation techniques have enabled efficient extraction of proteins and peptides from microorganisms, optimizing factors like nutrient supply, pH control, and oxygenation (Titiri *et al.*, 2023).

(D) Marine sources:

Marine sources also play a significant role in providing proteins and peptides with diverse functionalities. Table 4 illustrates some explanations and examples.

Overall, marine sources offer a wide array of proteins and peptides with various functionalities. The diversity of marine organisms and their unique adaptations to the marine environment provide ample opportunities for discovering novel bioactive compounds for pharmaceutical, nutraceutical, and cosmetic applications.

Methods for Protein and Peptide Extraction:

Table 5 depicts extraction methods and their examples. Extraction methods are – (A) Chemical extraction methods, (B) Physical extraction

methods, (C) Enzyme-assisted extraction, and (D) Emerging extraction techniques.

Factors Influencing Extraction Efficiency:

(A) Influence of biological source characteristics:

This refers to the inherent properties of the biological material from which proteins and peptides are being extracted. Factors such as tissue type, cell wall composition, presence of secondary metabolites, and overall biochemical composition can impact the efficiency of extraction. Different biological sources may require different extraction methods or optimization strategies to effectively release proteins and peptides (Anal *et al.*, 2013).

(B) Impact of extraction conditions: Extraction conditions encompass various parameters such as pH, temperature, solvent composition, extraction time, and the presence of additives like detergents or chaotropic agents. These conditions can significantly influence the efficiency and selectivity of protein and peptide extraction. Optimizing extraction conditions is crucial to achieve high yields while maintaining the integrity and functionality of the extracted biomolecules (Mostashari *et al.*, 2023).

(C) Role of target protein or peptide properties:

This factor focuses on the specific characteristics of the proteins or peptides being targeted for

Table 4: Examples and methods of extraction of proteins and peptides for Marine sources

Marine Sources	Description	References
Micro-organism	Marine microorganisms, including bacteria, fungi, algae, and archaea, inhabit various marine environments such as the ocean, seabed, and coastal regions. For instance, marine bacteria like <i>Vibrio</i> species produce antimicrobial peptides with potential therapeutic applications. Marine fungi have been found to produce bioactive compounds such as marine fungal extracts containing antimicrobial peptides.	Alves <i>et al.</i> (2020)
Animals	Sponges are well-known for producing bioactive compounds, including proteins and peptides with potential medicinal uses. Fish, such as salmon and tuna, are rich in proteins, including essential amino acids, and are commonly consumed as food for their nutritional value.	Caruso <i>et al.</i> (2020)
Algae and Seaweeds	For instance, spirulina, a type of blue-green algae, is rich in proteins, essential amino acids, antioxidants, and other bioactive peptides, making it a popular dietary supplement. Seaweeds like kelp, nori, and wakame are also consumed and used in food products due to their protein content and potential health benefits.	Pandey <i>et al.</i> (2020)
By-products	Protein and peptide extraction from marine sources often involves utilizing byproducts generated during fish or seafood processing. For example, fish processing waste, such as, bones, and skin, can be sources of collagen proteins and peptides. Collagen peptides derived from marine sources have gained popularity for their applications in skincare and other industries	Rajabimashhadi <i>et al.</i> (2023)

extraction. Properties such as size, structure, hydrophobicity, stability, post-translational modifications, and abundance can affect their extractability. Understanding these properties is essential for selecting appropriate extraction methods and optimizing protocols to maximize extraction efficiency and preserve the integrity of the target biomolecules (Reeves, 2010).

Applications of Extracted Proteins and Peptides:

(i) Food and beverage industry: Proteins and peptides extracted from biological sources find numerous applications in the food and beverage industry. They can be utilized as functional ingredients to improve the texture, flavour, and nutritional profile of food products. For example, proteins such as casein and whey are commonly used in dairy products for their emulsifying and

gelling properties. Peptides derived from plant or animal sources may also be incorporated into food formulations for their bioactive properties, such as antioxidant, antimicrobial, or health-promoting effects (Roy *et al.*, 2023).

(ii) Pharmaceutical and medical applications: Extracted proteins and peptides play crucial roles in pharmaceutical and medical applications. They serve as active pharmaceutical ingredients (APIs) in drug formulations, where they can act as therapeutic agents or drug carriers. Peptides, in particular, are increasingly utilized in drug discovery and development due to their specificity, potency, and lower toxicity compared to small-molecule drugs. Proteins and peptides derived from biological sources also have applications in regenerative medicine, tissue

Table 5: Extraction Methods and their examples

Extraction Method	Examples
A. Chemical Extraction	
Organic solvent extraction (Bourgou <i>et al.</i> , 2021)	- Extraction of lipids from plant seeds using hexane. - Isolation of proteins from animal tissues using acetone precipitation. - Extraction of membrane proteins using chloroform-methanol mixture. - Ethanol precipitation method for the extraction of nucleic acids from cell lysate - Isolation of hydrophobic proteins using a mixture of ethanol and ether.
Detergent-based extraction (Das and Negi, 2024)	- Solubilization of membrane proteins using Triton X-100. - Extraction of integral membrane proteins using sodium dodecyl sulphate (SDS). - Isolation of nuclear proteins using NP-40 detergent. - Extraction of membrane-bound enzymes using digitonin. - Purification of membrane vesicles using octyl glucoside.
Acidic extraction (Liu <i>et al.</i> , 2023)	- Extraction of histones using hydrochloric acid (HCl). - Acid precipitation method for the removal of albumin from serum samples. - Solubilization of collagen from connective tissues using acetic acid. - Extraction of plant cell wall proteins using sulfuric acid. - Acidic extraction of ribosomal proteins from cells using perchloric acid.
Salting-out extraction (Suman and Yugandhar, 2024)	- Precipitation of proteins using ammonium sulphate. - Fractionation of proteins based on solubility using sodium chloride. - Isolation of DNA-binding proteins using ammonium sulphate precipitation. - Salt-assisted extraction of intracellular proteins using potassium phosphate buffer. - Purification of enzymes using ammonium sulphate fractionation.
Metal-based extraction (Holte <i>et al.</i> , 2023)	- Immobilized Metal Affinity Chromatography (IMAC) for purification of His-tagged proteins. - Zinc chloride precipitation for isolation of metalloproteins. - Nickel-nitrilotriacetic acid (Ni-NTA) chromatography for purification of recombinant proteins. - Extraction of iron-binding proteins using iron chloride. - Copper sulphate precipitation for removal of albumin from serum samples.
B. Physical Extraction	
Homogenization (Zweng <i>et al.</i> , 2023)	- High-speed blender homogenization for plant tissue extraction. - Bead beating method for microbial cell lysis. - French press homogenization for bacterial cell disruption. -Tissue grinder homogenization for animal tissue extraction. - Ultrasonic homogenization for small-scale protein extraction.

Extraction Method	Examples
Sonication (Gokulakrishnan <i>et al.</i> , 2023)	• Bath sonication for microbial protein extraction. • Probe sonication for cell disruption and release of intracellular proteins. • Flow cell sonication for continuous protein extraction. • Water bath sonication for solubilization of membrane proteins. • Microplate sonication for high-throughput protein extraction.
Freeze-thaw cycling (Gokulakrishnan <i>et al.</i> , 2023)	• Freeze-thaw lysis for bacterial cell disruption. • Freeze-thaw extraction for release of membrane proteins. • Freeze-thaw cycling for protein release from animal tissues. • Freeze-thaw lysis for disruption of plant cell walls. • Freeze-thaw extraction for extraction of intracellular proteins.
Pressure-based extraction (Lee <i>et al.</i> , 2024)	• High-pressure homogenization for microbial protein extraction. • Pressure cycling technology for rapid protein extraction. • High-pressure cell disruption for recombinant protein production. • High-pressure syringe homogenization for tissue lysis. • -Pressure-driven cell disruption for protein purification.
Grinding/milling (Evangelista <i>et al.</i> , 2021)	• Cryogenic grinding for plant tissue protein extraction. • Ball milling for disruption of microbial cells. • Mortar and pestle grinding for tissue homogenization. • Vibratory disc mill for extraction of proteins from seeds. • Knife mill grinding for isolation of proteins from food samples.
C. Enzyme-assisted Extraction	
Trypsin digestion (Bailey <i>et al.</i> , 2024)	• Trypsin digestion for protein identification in proteomics. • In-gel trypsin digestion for protein identification in gel electrophoresis. • Trypsin digestion for peptide mapping. • Trypsin digestion for peptide library generation of protein sequencing.
Pepsin digestion (Loveday, 2022)	• Pepsin digestion for food protein hydrolysis. • Pepsin digestion for collagen extraction. • Pepsin digestion for peptide synthesis. • Pepsin digestion for protein fragmentation. • Pepsin digestion for peptide mapping.
Cellulase treatment (Khan <i>et al.</i> , 2023)	• Cellulase treatment for plant protein extraction. • Cellulase treatment for biomass protein isolation. • Cellulase treatment for cellulose-free protein extraction. • Cellulase treatment for plant cell wall protein analysis. • Cellulase treatment for plant protein crystallization.
Lytic enzyme cocktails (Singh <i>et al.</i> , 2023)	• Lysozyme and mutanolysin cocktail for bacterial cell lysis. • Cellulase and hemicellulose cocktail for plant cell wall disruption. • Protease and lipase cocktail for tissue protein extraction. • Chitinase and glucanase cocktail for fungal cell lysis. • Pectinase and cellulase cocktail for fruit protein extraction.

Extraction Method	Examples
Lipase treatment (Nimkande <i>et al.</i> , 2023)	• Lipase treatment for lipid-rich sample protein extraction. • Lipase treatment for membrane protein isolation. • Lipase treatment for detergent removal in protein purification. • Lipase treatment for milk protein extraction. • Lipase treatment for oilseed protein isolation.
D. Emerging Extraction Techniques	
Ultrasound-assisted extraction (Zhang <i>et al.</i> , 2024)	• Ultrasound-assisted extraction for plant protein isolation. • Ultrasound-assisted extraction for marine protein extraction. • Ultrasound-assisted extraction for intracellular protein release. • Ultrasound-assisted extraction for microbial protein isolation. • Ultrasound-assisted extraction for food protein recovery.
Microwave-assisted extraction (André <i>et al.</i> , 2023)	• Microwave-assisted extraction for plant protein extraction. • Microwave-assisted extraction for microbial protein isolation. • Microwave-assisted extraction for tissue protein extraction. • Microwave-assisted extraction for food protein recovery. • Microwave-assisted extraction for marine protein isolation.
Reverse micelle extraction (Zhao <i>et al.</i> , 2023)	• -Reverse micelle extraction for membrane protein isolation. • Reverse micelle extraction for hydrophobic protein isolation.

engineering, and diagnostic assays (Rostami *et al.*, 2023).

(iii) Biotechnological and industrial applications:

Proteins and peptides extracted from biological sources have diverse biotechnological and industrial applications. They are used in biocatalysis, where enzymes derived from proteins facilitate chemical reactions in various industrial processes, such as food processing, biofuel production, and pharmaceutical synthesis. Additionally, proteins and peptides are employed as bioremediation agents to degrade pollutants and contaminants in environmental cleanup efforts. In the field of biotechnology, proteins and peptides are crucial components of biologics, including therapeutic antibodies, vaccines, and recombinant proteins produced through genetic engineering techniques (Chauhan and Kanwar, 2020).

Challenges and Future Perspectives:

(A) Current challenges in protein and peptide extraction:

Efficiency and Yield: Despite advancements in extraction techniques, achieving high extraction efficiency and yield remains a challenge, particularly for low-abundance or complex proteins and peptides.

Selectivity: Selective extraction of target proteins and peptides while minimizing co-extraction of unwanted contaminants is challenging, especially from complex biological matrices.

Preservation of Biomolecule Integrity: Maintaining the integrity, stability, and functionality of extracted proteins and peptides during extraction processes is crucial but can be difficult, especially for fragile or sensitive biomolecules.

Standardization: Lack of standardized protocols and variability in extraction conditions hinder reproducibility and comparability of results across different studies and laboratories.

Scalability: Scaling up extraction processes from laboratory-scale to industrial-scale remains a challenge, requiring optimization of extraction

techniques and equipment to meet large-scale production demands.

(B) Potential advancements and areas for future research:

Development of Innovative Extraction Techniques:

Continued research into novel extraction methods, such as advanced chromatographic approaches, enzymatic digestion strategies, and emerging technologies like ultrasound-assisted extraction or microfluidics, can improve efficiency, selectivity, and scalability of protein and peptide extraction.

Integration of Omics Technologies: Integration of omics technologies, including genomics, proteomics, and metabolomics, can provide valuable insights into the composition, structure, and interactions of proteins and peptides within biological sources, guiding the development of targeted extraction approaches.

Bioinformatics and Machine Learning: Leveraging bioinformatics tools and machine learning algorithms for data analysis and prediction can aid in the optimization of extraction protocols, identification of target biomolecules, and characterization of extraction efficiency.

Sustainable Extraction Practices: Research into sustainable extraction methods utilizing green solvents, renewable resources, and eco-friendly processes can reduce environmental impact and promote the development of environmentally sustainable protein and peptide extraction techniques.

Standardization and Quality Control:

Establishment of standardized protocols, reference materials, and quality control measures for protein and peptide extraction will enhance reproducibility, reliability, and comparability of results across different studies and applications.

Conclusion

In conclusion, protein and peptide extraction from biological sources are vital processes with widespread applications in various industries, including food, pharmaceuticals, and biotechnology. While significant progress has been made

in extraction techniques, numerous challenges remain, including efficiency, selectivity, scalability, and standardization. Addressing these challenges requires interdisciplinary efforts, innovative research approaches, and collaboration between academia, industry, and regulatory bodies. Future advancements in extraction technology, coupled with advancements in omics, bioinformatics, and sustainability, hold promise for overcoming current limitations and unlocking the full potential of protein and peptide extraction for scientific, industrial, and societal benefit.

Acknowledgements

The authors gratefully acknowledge the facility provided by SMBT College of Pharmacy to prepare this manuscript

Conflict of Interest

The authors declare no conflicts of interest.

References

- Ali Al Jabr F, Mossad AS, Ali SAZ, Mohammad IAH, Husain AAT, Ali Mohammed AQ. (2020) Red and white cabbage extracts: Antioxidant effects on bovines albumins. *Int J Pharm Res Allied Sci.* 9(3): 97-104.
- Alves A, Emilia S, Kijjoa A and Pinto M. (2020) Marine-derived compounds with potential use as cosmeceuticals and nutricosmetics. *Molecules* 25(11): 2536.
- Anal AK, Noomhorm A and Vongsawasdi P. (2013) Protein hydrolysates and bioactive peptides from seafood and crustacean waste: Their extraction, bioactive properties and industrial perspectives. In: *Marine Proteins and Peptides*, (ed.) Se-Kwon K., John Wiley & Sons, Ltd, pp. 709-735
- André J, Flórez-Fernández N, Domínguez H and Torres MD. (2023) Microwave-assisted extraction of *Ulva* spp. including a stage of selective coagulation of ulvan stimulated by a bio-ionic liquid. *Int J Biol Macromol* 225: 952-963.
- Arrutia F, Eleanor B, Peter W and Keith WW. (2020) Oilseeds beyond oil: Press cakes and meals supplying global protein requirements. *Trends Food Sci Technol* 100: 88-102.
- Bailey HJ, Jonathan E, Joshua V, Eva-Maria L, Ivan K, Tetiana M, Nataliya T, Julian DL, Ansgar AW, Fiona JS and Ivan D. (2024) Engineering CRBN for rapid

- identification of next generation binders. bioRxiv. <https://doi.org/10.1101/2024.01.18.576231>
- Bilal M and Iqbal HMN. (2020) Biologically active macromolecules: Extraction strategies, therapeutic potential and biomedical perspective. *Int J Biol Macromol* 151: 1-18.
- Bourgou S, Bettaieb Rebey I, Ben Kaab S, Hammami M, Dakhlaoui S, Sawssen S, Msaada K, Isoda H, Ksouri R and Fauconnier ML. (2021) Green solvent to substitute hexane for bioactive lipids extraction from black cumin and basil seeds. *Foods* 10(7): 1493.
- Caruso G, Floris R, Serangeli C and Di Paola L. (2020) Fishery wastes as a yet undiscovered treasure from the sea: Biomolecules sources, extraction methods and valorization. *Mar Drugs* 18(12): 622.
- Chauhan V and Kanwar SS. (2020) Bioactive peptides: Synthesis, functions and biotechnological applications. In: *Biotechnological Production of Bioactive Compounds*, (eds.) Verma M.L. and Chandel A.K., Elsevier, pp. 107-137.
- Cheran E, Sharmila Rahale C, Divyabharathi P, Viswanathan C and Narayanan L. (2024) Corn cob nanocellulose packaging for increasing the shelf life of food products. *Int J Biol Macromol* 268(Pt 1): 131403.
- Das S and Negi S. (2024) A novel strategy for partial purification of alkane hydroxylase from *P. chrysogenum* SNP5 through reconstituting its native membrane into liposome. *Sci Rep*. 14: 3779.
- Deleu Lomme J, Lemmens E, Redant L and Dekour J. (2020) The major constituents of rye (*Secale cereale* L.) flour and their role in the production of rye bread, a food product to which a multitude of health aspects are ascribed. *Cereal Chem*. 97: 739-754.
- Dewi KR, Ismayati Maya, Solihat NN, Dewi NY, Kusnandar F, Riantana H, Heryani H, Halim A, Acter T, Uddin N and Kim S. (2023) Advances and key considerations of liquid chromatography-mass spectrometry for porcine authentication in halal analysis. *J Anal Sci Technol* 14: 13.
- Ermakova GV, Meyntser IV, Zaraisky AG and Bayramov AV. (2024) Loss of noggin1, a classic embryonic inducer gene, in elasmobranchs. *Sci Rep*. 14(1): 3805.
- Escher BI, Matthew JB, König M, Lei YD and Wania F. (2023) Mixture effect assessment applying in vitro bioassays to in-tissue silicone extracts of traditional foods prepared from beluga whale blubber. *Environ Sci Process Impacts* 25: 1759-1770.
- Evangelista R, Hojilla-Evangelista MP, Cermak SC and Van Tassel DL. (2021) Physical properties and processing of *Silphium integrifolium* seeds to obtain oil and enriched protein meal *J American Oil Chem Soc*. 100(1): 81-89.
- Ghoshal G and Kaur M. (2023) Optimization of extraction of starch from sweet potato and its application in making edible film. *Food Chem Adv*. 3: 100356.
- Gianazza E, Ivano E, Luca P and Ingrid M. (2021) Hemolymph proteins: An overview across marine arthropods and molluscs. *J Proteomics* 245: 104294.
- Gokulakrishnan B, Praveen S, Subash B, Karthick K, Sakthivel B, Sanjay T C and Balasubramanian M. (2023) Fabrication of ultra sonic probe sonicator – A review. *Int J Trendy Res Engineer technol*. 7(6): 1-4.
- Haller, N. (2023) Separation of the major whey proteins α -Lactalbumin and β -Lactoglobulin by means of selective precipitation and continuous centrifugal separation. Dissertation, TUM School of Life Sciences, Universität München.
- Halm D. (2024) Neglected tropical diseases and long-term captive animals: Ethical considerations with venom lab snakes. *J Appl Anim Ethics Res*. 1: 1-22.
- Higa F, House JD and Nickerson MT. (2024) Functionality and nutritional properties of yellow pea, green lentil, chickpea, and navy bean proteins extracted by different methods. *European Food Res Technol* 250: 273-286.
- Holte C, Szafranska K, Kruse L, Simon-Santamaria J, Li R, Svistounov D and McCourt P. (2023) Highly oxidized albumin is cleared by liver sinusoidal endothelial cells via the receptors stabilin-1 and -2. *Sci Rep*. 13(1): 19121.
- Hu FB. (2003) Plant-based foods and prevention of cardiovascular disease: An overview. *Am J Clin Nutr*. 78: 544S-551S.
- Huang S, Rao Y, Ju AL, Ker DFE, Blocki AM, Wang DM and Tuan RS. (2024) Non-collagenous proteins, rather than the collagens, are key biochemical factors that mediate tenogenic bioactivity of tendon extracellular matrix. *Acta Biomater*. 176: 99-115.
- Indriani S, Supatra K, Nilesh PN and Sithipong N. (2023) Amphibian skin and skin secretion: An exotic source of bioactive peptides and its application. *Foods* 12(6): 1282.
- Islam F, Amer Ali Y, Imran A, Afzaal M, Zahra SM, Fatima M, Saeed F, Usman I, Shehzadi U, Mehta S and Shah MA. (2023) Vegetable proteins as encapsulating agents: Recent updates and future perspectives. *Food Sci Nutr*. 11(4): 1705-1717.
- Jaeger A, Zannini E, Sahin AW and Arendt EK. (2021) Barley protein properties, extraction

- and applications, with a focus on Brewers' spent grain protein. *Foods* 10(6): 1389.
- Janssen F, Courtin CM and Wouters AGB. (2024) Aqueous phase extractable protein of wheat bran and germ for the production of liquid and semi-solid foods. *Crit Rev Food Sci Nutr*. 64(26): 9585-9603.
- Jawed A. (2023) Biomolecules: Types, homogenization, bead beater, sonication. In: *Basic Biotechniques for Bioprocess and Bioentrepreneurship*, (eds) Bhatt A.K., Bhatia R.K. and Bhalla T.C., Academic Press, pp. 125-131.
- Kamal H, Cheng FL, Andrew MS and Asgar A. (2021) Extraction of protein from food waste: An overview of current status and opportunities. *Compr Rev Food Sci Food Saf*. 20: 2455-2475.
- Khan GA, Dutta A, van de Meene A, Frandsen KEH, Ogden M, Whelan J and Persson S. (2024) Phosphate starvation regulates cellulose synthesis to modify root growth. *Plant Physiol*. 194(2): 1204-1217.
- Kheiralipour K, Rafiee S, Karimi M, Nadimi M and Paliwal J. (2023) The environmental impacts of commercial poultry production systems using life cycle assessment: a review. *World's Poultry Sci J*. 80(1): 33-54.
- Kumar M, Tomar M, Potkule J, Reetu, Punia S, Dhakane-Lad J, Singh S, Dhumal S, Pradhan PC, Bhushan B, Anitha T, Alajil O, Alhariri A, Amarowicz R, Kennedy JF and Laihonon L. (2024) Transcriptomic and proteomic analyses of distinct *Arabidopsis* organs reveal high PSI-NDH complex accumulation in stems. *Physiol Plant* 176: e14227.
- Lee S, Kim E, Jo M and Choi YJ. (2024) Characterization of yeast protein isolates extracted via high-pressure homogenization and pH shift: A promising protein source enriched with essential amino acids and branched-chain amino acids. *J Food Sci*. 89(2): 900-912.
- Liu S, Li Z, Yu B, Wang S, Shen Y and Cong H. (2020) Recent advances on protein separation and purification methods. *Adv Colloid Interface Sci*. 284: 102254.
- Liu Z, Wu Y, Mao X, Kwan KCJ, Cheng X, Li X, Jing Y and Li XD. (2023) Development of multifunctional synthetic nucleosomes to interrogate chromatin-mediated protein interactions. *Sci Adv*. 9(18): eade5186.
- Llauger M, Guerrero L, Arnau J, Morera A, Wakamatsu JJ, Lorenzo JM and Bou R. (2024) Zinc protoporphyrin-rich pork liver homogenates as coloring ingredients in nitrite-free liver pâtés. *Foods* 13(4):533.
- Loveday SM. (2023) Protein digestion and absorption: the influence of food processing. *Nutr Res Rev*. 36(2): 544-559.
- Lu WC, Chien-Shan C, Yung-Jia C, Tresiliana MA and Po-Hsien L. (2023) Characterization and biological properties of marine by-product collagen through ultrasound-assisted extraction. *Aquacult Rep*. 29: 101514.
- Lu X, Chang R, Lu H, Ma R, Qiu L and Tian Y. (2021) Effect of amino acids composing rice protein on rice starch digestibility. *LWT* 146: 111417.
- Mahdavi Yekta M, Nouri L, Azizi MH, Karimi Dehkordi M, Mohammadi M, Jabbari M, Rezaei M and Mousavi Khaneghah A. (2020) Peptide extracted from quinoa by pepsin and alkalase enzymes hydrolysis: evaluation of the antioxidant activity. *J Food Process Preserv*. 44(10): e14773.
- Mamone G and Picariello G. (2023) Optimized extraction and large-scale proteomics of pig jejunum brush border membranes for use in in vitro digestion models. *Food Res Int*. 164: 112326.
- Mazumder S and Zhang N. (2023) Cellulose--hemicellulose--lignin interaction in the secondary cell wall of coconut endocarp. *Biomimetics* 8: 188.
- McCabe MC, Saviola AJ and Hansen KC. (2023) Mass spectrometry-based atlas of extracellular matrix proteins across 25 mouse organs. *J Proteome Res*. 22(3): 790-801.
- Meyllianawaty PF, Haetami K and Musthopa AA. (2024) Interventions in selection of fish feed ingredients with special reference to leaves and water plants: a review. *Asian J Fish Aquat Res*. 26(1): 88-95.
- Mondal K and Katiyar V. (2024) Pectin from agro-waste residues for food packaging. In: *Agro-Waste Derived Biopolymers and Biocomposites: Innovations and Sustainability in Food Packaging*, (eds.) Kumar S., Mukherjee A. and Katiyar V., Wiley, pp. 319-348.
- Mostashari P, Marszałek K, Aliyeva A and Mousavi Khaneghah A. (2023) The impact of processing and extraction methods on the allergenicity of targeted protein quantification as well as bioactive peptides derived from egg. *Molecules* 28(6): 2658.
- Mourad FK, Sijie M, Haoyang S, Cuie T, Long S and Cai Z. (2023) Development of stable organogels using egg yolk granules-chitosan complex: Structure, rheology, and stability. *Colloids Surfaces A Physicochem. Eng. Asp*. 676: 132206.
- Nimkande VD, Krishnamurthi K and Bafana A. (2023) Potential of antarctic lipase from *Acinetobacter johnsonii* Ant12 for treatment of lipid-rich wastewater: screening, production, properties and applications. *Biodegradation* 34(6): 549-566.
- Pandey AK, Chauhan OP and Semwal AD. (2020) Seaweeds – a potential source for functional foods.

- Peluso P and Chankvetadze B. (2024) Recent developments in molecular modeling tools and applications related to pharmaceutical and biomedical research. *J Pharm Biomed Anal* 238: 115836.
- Prade T, Faraz M, Emilia B, Anna-Lovisa N, Sven-Erik S, William RN and Eva J. (2021) Protein fractionation of broccoli (*Brassica oleracea*, var. Italica) and kale (*Brassica oleracea*, var. Sabellica) residual leaves — A pre-feasibility assessment and evaluation of fraction phenol and fibre content. *Food Bioprod Process* 130: 229-243.
- Rajabimashhadi Z, Gallo N, Salvatore L and Lionetto F. (2023) Collagen derived from fish industry waste: progresses and challenges. *Polymers (Basel)* 15(3): 544.
- Ramondo A, Serena M, Angela S, Paolo M and Prospero DP. (2024) Modification of physicochemical and functional properties of pumpkin seeds protein isolate (PsPI) by high-intensity ultrasound: effect of treatment time. *ACS Food Sci Technol* 4: 40-48.
- Rana D, Desai N, Salave S, Karunakaran B, Giri J, Benival D, Gorantla S and Kommineni N. (2023) Collagen-based hydrogels for the eye: A comprehensive review. *Gels* 9(8): 643.
- Rebouças JSA, Oliveira FPS, Araujo ACS, Gouveia HL, Latorres JM, Martins VG, Prentice Hernández C and Tesser MB. (2023) Shellfish industrial waste reuse. *Crit Rev Biotechnol* 43(1): 50-66.
- Reeves A. (2010) The importance of an overview. *Couns Psychother Res*. 10: 237.
- van Rensburg W. (2024) Manipulation of the rumen environment to increase rumen undegradable protein fraction of feedstuffs. Masters Thesis, Agricultural Sciences (Animal Science), Faculty AgriSciences, Stellenbosch University.
- von Reumont BM, Dutertre S and Koludarov I. (2022) Venom profile of the European carpenter bee *Xylocopa violacea*: Evolutionary and applied considerations on its toxin components. *Toxicon* X. 14: 100117.
- Rostami M, Beheshtizadeh N, Ranjbar FE, Najafi N, Ahmadi A, Ahmadi P, Rostamabadi H, Pazhouhnia Z, Assadpour E, Mirzanajafi-Zanjani M, Kisomi MF, Kharazmi MS and Jafari SM. (2023) Recent advances in electrospun protein fibers/nanofibers for the food and biomedical applications. *Adv Colloid Interface Sci*. 311: 102827.
- RoyT, Singh A, Sari TP and Homroy S. (2023) Rice protein: Emerging insights of extraction, structural characteristics, functionality, and application in the food industry. *J Food Compos Anal* 123: 105581.
- Shi T, Cao J, Cao J, Zhu F, Cao F and Su E. Almond (*Amygdalus communis* L.) kernel protein: A review on the extraction, functional properties and nutritional value. *Food Res Int*. 167: 112721.
- Si J, Gao Y, Yan B, Shen X, Changfeng Z and Yao Q. (2024) Evaluating the impact of delayed centrifugation on protein profiles analyzed by LC/MS in serum and plasma samples. *Adv Sens Energy Mater*. 3: 100101.
- Singh B, Soni SK, Garg N and Mathur P. (2023) Precise utilization of fruit and vegetable waste as a substrate for the multienzyme production by *Bacillus subtilis* NG105 and *Trichoderma asperellum* NG125. *Biocatal Agricult Biotechnol* 51: 102797.
- Solomon W, Mutum L, Janda T and Molnar Z. (2023) Potential benefit of microalgae and their interaction with bacteria to sustainable crop production. *Plant Growth Regul* 101: 53-65.
- Steinbruch E, Wise J, Levkov K, Chemodanov A, Israel Á, Yoav DL and Alexander G. (2023) Enzymatic cell wall degradation combined with pulsed electric fields increases yields of water-soluble-protein extraction from the green marine macroalga *Ulva* sp. *Innov Food Sci Emerg Technol* 84: 103231.
- Suman V and Yugandhar NM. (2024) partial purification and characterization of pectinase from Actinomycete: *Nocardia dasdonovelli* isolated from marine samples. <https://doi.org/10.21203/rs.3.rs-3922915/v>
- Thery T, Kieran ML, Emanuele Z and Elke KA. (2020) Isolation, characterisation and application of a new antifungal protein from broccoli seeds – New food preservative with great potential. *Food Control* 117: 107356.
- Titiri E, Filippi K, Giannakis N, Vlysidis A, Koutinas A and Stylianou E. (2023) Optimisation of alkaline pretreatment of spent coffee grounds for microbial oil production by *Cryptococcus curvatus*. *Biochem Eng J*. 193: 108841.
- Trigo JP, Engström N, Steinhagen S, Juul L, Harrysson H, Toth GB, Pavia H, Scheers N and Undeland I. (2021) In vitro digestibility and Caco-2 cell bioavailability of sea lettuce (*Ulva fenestrata*) proteins extracted using pH-shift processing. *Food Chem*. 356: 129683.
- Umaru IJ, Michael SA, Umaru HA and Umaru KI. (2024) Aqueous enzymatic extraction of oil In: *Enzymes in Oil Processing*, (eds.) Showkat A.B., Khan A., Awang A.S. A.H. and Mohd R.A., Elsevier, pp. 169-185.
- Valente AI, Ferreira AM, Almeida MR, Mohamadou A, Freire MG and Tavares APM. (2022) Efficient extraction of the RuBisCO enzyme from spinach

- leaves using aqueous solutions of biocompatible ionic liquids. *Sustain Chem.* 3: 1-18.
- Wang R, Guo F, Zhao J and Feng C. (2024) Myofibril degradation and structural changes in myofibrillar proteins of porcine longissimus muscles during frozen storage. *Food Chem.* 435: 137671.
- Xu H, Hao Q, Liu H, Lulu C, Ran W, Liang Q, Hua G, Jinrong L, Chenyu Y, Hao H, Kun X, Jinchao F, Yijun Z, Biao L, Gaopeng L and Xiaodong W. (2023) A concentration-descending washing strategy with methanol for the enhancement of protein imaging in biological tissues by MALDI-MS. *Analyst* 148: 823-831.
- Yu Y, Kleuter M, Taghian Dinani S, Trindade LM and van der Goot AJ. (2023) The role of plant age and leaf position on protein extraction and phenolic compounds removal from tomato (*Solanum lycopersicum*) leaves using food-grade solvents. *Food Chem.* 406: 135072.
- Zhang W, Isaac DB and Jinsheng X. (2024) Novel marine proteins as a global protein supply and human nutrition: Extraction, bioactivities, potential applications, safety assessment, and deodorization technologies. *Trends Food Sci Technol* 143: 104283.
- Zhao Y, Tian R, Xu Z, Jiang L and Sui X. (2023) Recent advances in soy protein extraction technology. *J Am Oil Chem Soc.* 100: 187-195.
- Zweng S, Mendoza-Rojas G, Lepak A and Altegoer F. (2025) Simplifying recombinant protein production: Combining golden gate cloning with a standardized protein purification scheme. *Methods Mol Biol* 2850: 229-249.