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Preface

Oceans of the world provide a bounty in the form of seafood to the human nutritional realm providing an array of food, biofunctional and biomedically significant proteins. The structural proteins being the fundamental units of the fish tissue provides a diverse drape of functional properties enabling them to be used for specific applications. Study on seafood structural proteins has garnered momentum recently owing to their distinctive attributes and versatile functions. From the shiny scales of a sardine to sturdy muscles of a tuna, the seafood structural proteins demonstrate a noteworthy diversity, captivating the interest of innovators and researchers worldwide. This book is a collective endeavour by a group of researchers who are interested in seafood structural protein functionality and applications, at the same time committed towards the sustainable utilisation of the marine resources. Attempt has been made to emphasise the importance of sustainability by particularly highlighting research targeting seafood side streams. The target audience for this publication are the students, researchers and industry innovators who are into marine protein technology with an inclination towards application and sustainability. The book is divided into eight chapters. The primary chapter introduces the significance of the marine protein in general, highlighting the nutritional significance of seafood as a source of essential amino acids, omega-3 fatty acids, vitamins, and minerals. It also gives a structured information to the reader regarding the general classification and the potential towards the seafood protein-based products. The second chapter paves light on the chemistry of the seafood structural proteins such as myofibrillar protein and collagen, their chemistry, types, and structure. The physical and functional properties of these proteins, which qualifies for specific applications, are also discussed in detail. The third chapter enumerates the basic parameters involved and technologies available for extracting, isolating, and characterising the seafood structural proteins. The fourth chapter deals with the extraction techniques involved in obtaining the derivatives of seafood structural proteins and their properties. The fifth chapter introduces the intricacies of bioinformatics and computational tools applied in protein research and its significance particularly in studying seafood proteins. The sixth chapter gives the reader a comprehensive information about the different food and biomedical applications of seafood proteins and the specific functionalities that enable them to be explored for particular applications. The seventh chapter discusses the pharmaceutical and hydrogel applications of marine collagen which is highly significant in the light of finding high value applications for

the seafood side streams. The eighth and the final chapter deals with the state-of-the-art arena of seafood protein-based biomaterials, which finds application in medical field in the form of biocompatible scaffolds and allied fixtures. The authors through this book endeavour to present comprehensive information on seafood proteins specifically structural proteins that could be extracted predominantly from seafood side streams resulting in a much more sustainable utilisation of the available resources.

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Editors and Contributors

About the Editors

Maya Raman graduated in Botany and received her M.Sc. (Industrial Fisheries) and Ph.D. degrees from the School of Industrial Fisheries, Cochin University of Science and Technology, India. Her doctoral area of study was in Fish Biochemistry and Fish Processing Technology. She is presently working as Associate Professor and Head, Department of Food Science and Technology, Kerala University of Fisheries and Ocean Studies. She also worked as postdoc at Applied Nutrition and Food Chemistry, Centre for Chemistry and Chemical Engineering, Lund University with postdoctoral scholarship from Erasmus Mundus External Cooperation Windows Lot 15 Programme. She also worked as Women Scientist at Indian Institute of Technology Madras with the Women Scientist Scheme (WOS-A) Funding by Department of Science and Technology, India. She was selected as Young Student Speaker IFCON 2003 by Association of Food Scientists and Technologists, India. She also worked as faculty at Department of Food Science and Technology, MACFAST, as certified Technologist (Export Inspection Authority, India) at Koluthara Exports Limited, and as Research Officer at NGIL, India. She has 39 peer-reviewed publications to her credit and has published 3 books and 16 book chapters. She has 37 abstracts/extended abstracts presented at national and international symposia/conferences. Her area of expertise is fish biochemistry (collagen), fish processing technology, nutrition and health (cancer), nanotechnology, bioactive marine compounds, dietary fibres (cereal, vegetable, marine algae) and colon cancer (in vitro and in vivo), molecular modelling and dynamics, gut simulation techniques, drug encapsulation, hydrogels, antimicrobial biodegradable food wraps. She is a life member of AFST(I), SOFT(I), SASNET, member of ACS, and is a member of several other renowned organisations. She has also qualified NET conducted by ASRB in 2001. A patent on hydrogel from cyclic beta-glucan for cosmetics/food applications was granted in 2022. She is offering two online courses through NPTEL-SWAYAM.

Abhilash Sasidharan is currently pursuing his postdoctoral research at the Department of Biotechnology & Food Science, Norwegian University of Science & Technology (NTNU), Trondheim, Norway. He is also serving as Assistant Professor, Department of Fish Processing Technology, Kerala University of Fisheries & Ocean

Studies (KUFOS), Kerala, India. He did his Post Graduation in Industrial Fisheries from Cochin University of Science & Technology (CUSAT), Kerala, India in 2003 and Ph.D. under the Faculty of Marine Sciences, CUSAT in 2014. His Ph.D. thesis focused on seafood side stream management and valorisation aspects. He is also recipient of the Young Scientist Fellowship of Department of Science & Technology in 2007 and was successful in developing a foliar fertilizer from seafood side stream which later resulted in developing a Startup called Green Allies Organics Pvt. Limited in 2012. He also worked as the State Coordinator & Thematic Expert of the Reliance Foundation Information Services, the CSR wing of the Reliance Industries Ltd., Research Associate, ZTMC-BPD, CIFT and as Senior Research Fellow in various R&D Projects in CIFT. He has about 49 peer-reviewed national and international publications, 5 book chapters and several seminar proceedings to his credit. His fields of interests are seafood side stream valorisation, seafood product and by-product technology, seafood packaging, thermal processing etc. He has also conducted several training programs and invited talks on various aspects of fish processing and seafood entrepreneurship topics for students and stakeholders.

S. Sabu Associate Professor in Fish Post Harvest Technology and the Director of the School of Industrial Fisheries of Cochin University of Science and Technology (CUSAT), graduated from the University of Kerala with a triple main degree (Biochemistry, Zoology and Industrial Fish and Fisheries) in the year 2001.Obtained his Masters' degree in Industrial fisheries from CUSAT in 2003 and completed his Ph.D. in Marine Science at the Indian Council for Agricultural Research-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin and awarded the doctoral degree by CUSAT in the year 2009. Qualified National Eligibility Test (s) (NET) in (1) Fish Harvest and Post Harvest Technology in 2009; (2) Fish Process Technology in 2014 conducted by the ICAR-Agricultural Scientists Recruitment Board (ASRB) of India. Before joining the CUSAT service, he possesses 5 years of UG teaching and administrative experience at the Govt of India, Central Institute of Fisheries Nautical and Engineering Training (CIFNET), Cochin. Industrial experience of S. Sabu includes one year of experience in seafood processing and quality assurance from Abad CAP Seafoods Pvt. Ltd, Cochin and two years of industrial chitin-chitosan manufacturing & quality assurance experience as the advisor of the P. T. Biotech Surindo, West Java, Indonesia. He also possesses one year of postdoctoral experience in the management & commercialization of technologies of 22 ICAR—Agriculture, Horticulture, Crop Science and Fisheries institutes in south India. He was a recipient of an international award from the World Wide Fund (WWF) for Nature, USA, in 2005 and the Best Scientific Paper award from the Society of Fisheries Technologists, India (SOFT(I)) in 2012. So far, he has published 40 peer-reviewed Scopus/SCI-indexed publications, three books, five book chapters, and more than 30 conference presentations and, under his guidance, awarded 1 Ph.D., 3 MPhil and more than 30 PG dissertations to date. The current research areas are food waste management, chitin-chitosan, food safety, application of natural extractives, chitosan derivatives, and nano-chitosan in agriculture crops/food and aquaculture. He is a member of the Cochin University Senate, academic council, academic committee and Board of Studies of CUSAT.

Dhanya Pulikkottil Rajan currently holds the position of Assistant Professor at M.E.S. Asmabi College, Thrissur, India. Prior to this role, she served as a faculty at the School of Industrial Fisheries, Cochin University of Science and Technology (CUSAT), and St. Albert's College, Ernakulam. She earned her post-graduate degree in Industrial Fisheries from CUSAT and obtained a doctoral degree in Marine Sciences. Her doctoral research delved into investigating the impact of spice oleoresins on microbial decontamination and their potential application in enhancing the quality of tuna (*Euthynnus affinis*) during storage. Dhanya has been recognised for her academic contributions, earning accolades such as best paper presenter awards at prestigious events. Presently, she holds key roles including the coordinator of the Research Promotion Council, Nodal Officer of the Young Innovators Programme initiated by K-Disc, and Assistant-Nodal Officer of the Innovation and Entrepreneurship Development Cell (IEDC) at M.E.S. Asmabi College. Additionally, she is a Lifetime Member of the Society for Fishery Technologists (India). With a wealth of experience spanning a decade in teaching and four years dedicated to research in the field of fish processing, she is an invited resource person in the realm of marine sciences. Prior to her research pursuits, she served as a certified Technologist at CAP Seafoods, Kerala. Her research interests encompass biopreservation, safety and sensory enhancement of seafood products, value addition, and the study of microbial spoilage by psychotropic and histamine-forming bacteria. Her contributions extend beyond the confines of academia, with 17 research papers published in esteemed international and national journals, presentations at conferences, a published book, and numerous book chapters. Furthermore, she actively mentors research scholars specialising in fish processing and fishery microbiology.

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Part I

Structure, Function and Bioinformatics

Introduction to Fish Proteins

1

S. Sabu

Abstract

Seafood is reliably regarded as the most preferred and affordable animal protein source in their diets for a large number of populations around the world. Marine-derived foods have muscle proteins that are high in nutrients, easily absorbed, and have a well-balanced amino acid composition. It has been demonstrated that fish is essential to a healthy diet, helps prevent undernutrition and micronutrient deficiencies in developing nations, and promotes food security. In addition to being a significant source of highly beneficial dietary omega-3 fatty acids, aquatic foods also offer all vital amino acids, superior proteins, vitamins, and minerals. Most seafood proteins are more than 90% digestible, and the muscle proteins of seafood are rich in all essential amino acids. This chapter elaborates on the significance of seafood in the human diet and the classification of fish proteins and fish protein-based products.

Keywords

Fish proteins · Nutritional quality · PUFA · FPH · Surimi · Marine collagen

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1.1 Nutritional Significance of Seafood

The popularity of aquatic foods, particularly seafood, is growing due to its ability to ensure nutrition and food security. Apart from being a protein source, it's also a special and highly varied source of essential and bioavailable omega-3 fatty acids. The year 2020 saw an estimated 178 million tons of aquatic animal production worldwide. More than 157 million tons were consumed by humans, with the remaining 20 million tons going toward non-food applications, primarily the production of fishmeal and fish oil. The amount of aquatic animal foods consumed per person increased from 9 kg in 1961 to 20.5 kg in 2019. The world is currently consuming over five times as much as it did approximately sixty years ago (FAO 2022). Foods found in the ocean are essential for a balanced and healthy diet. Due to their ability to supply vital nutrients that are hard to come by in plant-based diets, even tiny amounts of aquatic foods can make a major nutritional difference. Fish is reliably regarded as the most preferred and affordable animal protein source for a large number of populations around the world in their diets. It is an excellent source of several important nutrients, such as high-quality fats and protein (macronutrients), vitamins, and minerals (micronutrients), all of which are crucial to the food and nutrition security of the globe.

Consumers are becoming more conscious of how food sources impact human health. Marine-derived foods have muscle proteins that are high in nutrients, easily absorbed, and have a well-balanced amino acid composition. It has been demonstrated that fish is essential to a healthy diet and that it helps prevent undernutrition and micronutrient deficiencies in developing nations, in addition to promoting food security (Kawarazuka and Bene 2011). As a chief basis of protein for 1/3rd of the world populace and as a popular healthy food, fish (including finfish, crustaceans, and mollusks) play a significant role in human nutrition. In addition to being an excellent source of protein and fat, fish, particularly the tiny native species, are highly nutrient-dense and may be essential in the fight against micronutrient-deficiency illnesses that are common in developing nations (Roos et al. 2003). In addition to being a major source of highly beneficial dietary omega-3 fatty acids, aquatic foods also offer all vital amino acids, superior proteins, vitamins (especially A, B, and D), and minerals *viz*., Ca, P, I, Zn, Mg, Fe, K, and Se (Lund 2013). Eating fish regularly lowers blood pressure, which in turn depresses the danger of stroke, further chronic illnesses, depression, and Alzheimer's (FAO 2022). Fish is an ideal food for both newborns and adults because it is a rich source of essential nutrients that are necessary for proper development (Abdollahi et al. 2021). Compared to most terrestrial meats, aquatic animal foods are higher in protein. Furthermore, Tacon and Metian (2013) suggest that amino acids like lysine and methionine, which are scarce in terrestrial meat proteins, are abundant in aquatic protein and highly digestible.

1.1.1 Nutritional Significance of Fish Proteins

Proteins made up of amino acids bound together by peptide bonds are thought to be the most versatile biomolecules (Pal et al. 2018). Proteins are the basis of many animal body structures (e.g. muscle, skin). They also form the enzymes that control chemical reactions throughout the body. The body needs proteins for growth and development, for the synthesis of hormones and enzymes, and for the maintenance and repair of aging tissues (Tacon et al. 2020). Most seafood proteins are more than 90% digestible, and the muscle proteins of seafood are rich in all essential amino acids. The chemical score of finfish is higher (70) compared to milk (60) and beef (69). Compared to beef (2.3) and milk proteins (2.5), the protein efficiency ratio (PER) is significantly higher at 3.5 (Venugopal 2018).

The nutritional value and sensory qualities of fish products are both influenced by proteins, making them essential components of fish meat. When describing the protein content of food items, crude protein is typically expressed as 6.25 N in composition data. Both proteins and NPN compounds fall under this category. The nitrogen present in some foods can be up to 25% nitrogen from the NPN (Sikorski et al. 1994). Fish quality and nutritional value are generally predicted by the chemical composition of the flesh (Ravichandran et al. 2011). The composition of fish, as stated by Love (1970), consists mainly of water, comprising 66–81%. Protein makes up 16–21%, while minerals account for 1.2–1.5% of the fish. Fat content ranges between 0.2 and 25%, and carbohydrates constitute 0–0.5%. When compared to mammalian protein, fish protein has a lower tryptophan content but is higher in amino acids like lysine and methionine (Begum et al. 2012). Fish provides more than 30% of the animal protein needed by about 60% of people in developing nations (Sujatha et al. (2013)). When compared to alternative protein sources like mutton, beef, and chicken, fish is generally thought to have a much greater satiety effect and a lower production cost per unit (Balami et al. 2019). In terms of commercial classification, seafood is generally divided into four groups: (1) below 10%; (2) 10 to 15%; (3) 15–20%; and (4) above 20%. This is based on the percentage of crude protein contained in the meat. Fish flesh from tunas has the highest protein content, at 26.41 ± 1.13%. Less protein is known to be present in marine invertebrates (Sikorski et al. 1994).

1.2 Classification of Fish Proteins

Muscles are classified as striated or smooth based on their structure and function. In vertebrates, striated muscle is further subdivided into cardiac and skeletal muscles. Fast and slow skeletal muscles are further subdivided into the skeletal muscle (Ochiai and Osawa 2020). In fish, the contraction of muscles is facilitated by skeletal muscle proteins. A variety of molecular, structural, contractile, and metabolic functions are exhibited by the fibrous diverse cell populations found in fish proteins. These cell populations also impact the growth rate and properties of muscle, including cohesiveness, and color, as well as the eventual functional properties of meat

(Sikorski et al. 2020; Ryu et al. 2021). Fish have shorter muscle fibers and less connective tissue than terrestrial animals. These muscles are divided into myotomes by the myocommata (fine connective tissue layers), which are essentially thin sheets that divide muscle fibers into ordered layers (Boland et al. 2018). Fish muscles are further divided into three main categories: major white muscles, superficial red muscles, and intermediate pink muscles. The axial muscle is made up primarily of fast white muscle fibers, with a sheet of pink midway muscle fibers lying in amongst them and a thin coating of peripheral muscle fibers that are slowly reddened (Bone et al. 1978). Fish muscle normally contains three main types of proteins: myofibrillar or contractile proteins, which make up roughly 60–65%; sarcoplasmic or enzymatic proteins, which make up 30–35%; and stroma or connective tissue proteins, which make up approximately 3–5% of the total protein content (Nowsad 2007). Based on the solubility in aqueous solutions, Huss (1995) divided the proteins found in fish muscle into three categories (Sarcoplasmic, Myofibrillar and Stroma or connective tissue proteins) (Fig. 1.1).

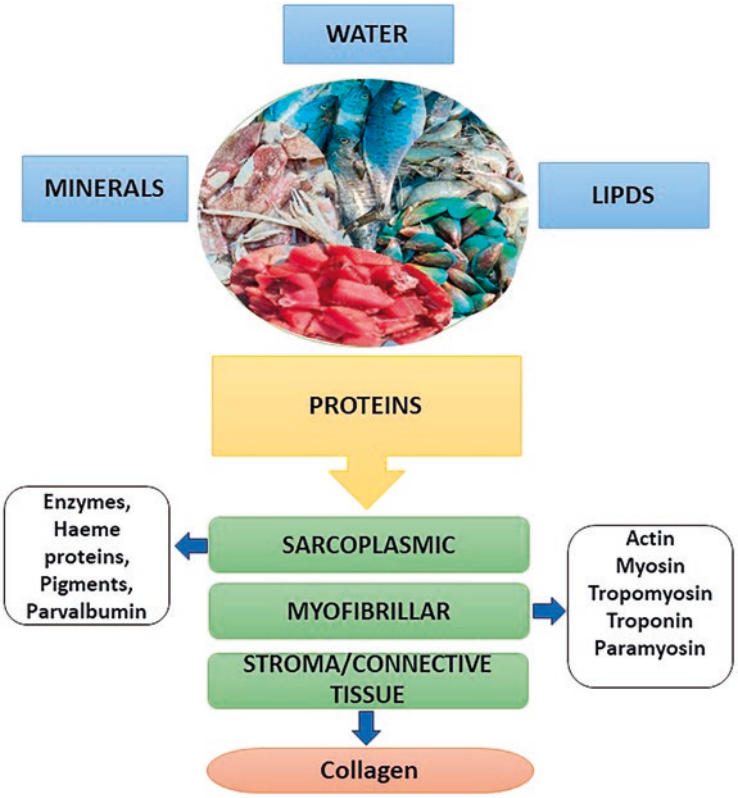

Fig. 1.1 Classification of seafood proteins

1.2.1 Sarcoplasmic Proteins

The sarcoplasmic fraction of fish muscle can dissolve in neutral salt solutions with an ionic strength of less than 0.15M. About 25–30% of the total proteins are made up of this fraction. In general, the meat of pelagic fish has a higher sarcoplasmic protein content than that of demersal fish, making up approximately 30% of the total protein content in Muscles. Hemoproteins make up a sizable portion of this protein fraction in the muscles of some animals (Sikorski et al. 1994). It is known that the sarcoplasmic fraction contains about 100 distinct proteins. The sarcoplasmic fraction of fish muscle comprises numerous varieties of protein components *viz*. Heme proteins, hemoglobin and myoglobin, as well as enzymes like aldolase, phospholipase, creatine kinase, proteases, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), transglutaminase, phosphorylase, peroxidase, beta-enolase, proteinase inhibitors, fructose-bisphosphate aldolase A, glycogen phosphorylase, triosephosphate isomerase B, phosphoglucomutase, phosphoglycerate kinase (Pazos et al. 2014; Vieira et al. 2018). In addition to enzymes, sarcoplasm contains pigments like myoglobin and the low molecular weight protein parvalbumin, both of which have a significant influence on the fish meat quality (Vieira et al. 2018).

1.2.2 Myofibrillar Proteins

Myofibrillar proteins, which make up 60–70% of all muscle proteins, are the long fibril proteins that are thought to be a major constituent of skeletal muscle. It is mostly involved in muscle contraction and is made up of thin components called actin and thick components called myosin (Dara et al. 2021). According to Sun and Holley (2011), myofibrillar proteins are crucial in the production of meat products with the necessary functional characteristic attributes. The myofibril proteins are soluble only in salt solutions (ionic strength above 0.5M). The process of extraction and the variables influencing the protein's condition determines the yield of this fraction, which is extracted from fresh meat (Sikorski et al. 1994). Muscle movement is attributed to the structural proteins that comprise the contractile apparatus. Although they may have slightly different physical characteristics, the amino acid composition is roughly the same as that of the corresponding proteins in mammalian tissue. Fish proteins change composition due to environmental changes. Okagaki et al. (2005) state that the two main types of muscle in fish are two main types of muscle in fish: (i) slow skeletal muscle, also known as dark muscle, and (ii) fast skeletal muscle, also known as ordinary muscle. To maintain osmoregulation, urea, a protein denaturant, accumulates in high concentrations in the tissues of sharks, rays, and lungfish. Some urea resistance is demonstrated by their myofibrillar proteins (Kanoh et al. 2000). The main component of myofibrillar proteins, myosin, affects the preservation of the three-dimensional structure of meat proteins and causes them to gel. The actin of myofibrillar protein acting as a critical part in strengthening the gel assembly of myosin. G-actin and F-actin are the two forms of the actin molecule and in the presence of salt, G-actin polymerizes into F-actin

(López-Bote 2017; Dara et al. 2021). The gelation of myosin is unaffected by the other myofibrillar protein components, such as tropomyosin and troponins. The actomyosin complex signifies the degree of meat tenderness through the interaction of myosin and actin (Wang et al. 2020).

1.2.2.1 Actin

Actin makes up about 20% of myofibrillar protein. When ATP is present, a globular (pear-shaped) monomer called G-actin polymerizes into a thin filament called F-actin that has a double helix and rosary-like conformation. Actin filaments are identified under a microscope as a light band (isotropic band, I band) in striated muscles. Actin is a multifunctional protein that, when combined with numerous partner proteins known as actin-binding proteins, is responsible for a wide range of cell motilities (Pollard 2016). Actin can be broadly classified into six isoforms: γ-type, which is present in smooth muscles, skeletal, and cardiac muscles; β-type, which is found in sarcoplasm; and γ-type, which is present in both sarcoplasm and smooth muscles (Simiczyjew et al. 2017: Ochiai and Osawa 2020).

1.2.2.2 Myosin

Of the total mass of protein in skeletal muscle, myosin makes up about 60%. Two hefty chain subunits, with about 200 kDa weights each, and four components of the light chain, weighing 20 kDa, make up the hexamer myosin fraction. As a result, the total molecular weight is almost 500,000. Myosin is classified into at least 15 classes, forming a superfamily based on its molecular structure and biological roles (Hartman and Spudich 2012). The ability of myosin to bind actin, form filaments, and exhibit ATPase activity varies among species and tissues. These properties can also be altered by adaptation to environmental factors, particularly temperature (Somero 2003). According to Okagaki et al. (2005), the slow skeletal (dark) and fast skeletal (ordinary) myosins of carp differ significantly from one another.

1.2.2.3 Tropomyosin

Tropomyosin, found in most vertebrates and many shellfish, is a dimer of 284 amino acid residue subunits. Across the whole molecule, its structure is typified by a parallel α-helical coiled coil. During physiological conditions, side-by-side polymerization between adjacent molecules at the N- and C-termini of each dimer results in the formation of a head-to-tail threadlike fibere (Sousa and Farah 2002; Ochiai et al. 2003; Ochiai and Osawa 2020). In contrast to the myosin-linked regulation of muscle contraction present in certain invertebrates, tropomyosin and troponin work together to regulate muscle contraction. In contrast to the striated muscles of mammals, which are made up of two different isoforms—β (TPM1) and β (TPM2)—the striated muscles of fish are homogeneous, containing only the α isoform (Perry 2001). With the added benefit of being highly soluble at low ionic strength, in contrast to myosin rods, tropomyosin is regarded as an exceptional prototypical protein for the study of the structure-stability association (Ochiai and Osawa 2020). When compared to tropomyosins from cold-water species, tropical fish species such as tilapia and milkfish tended to be more thermostable (Huang et al. 2019). Although

the number of tropomyosins in mollusks and crustaceans can be as low as a few milligrams per gram of muscle, they are known to be the main allergen. Conversely, for many, vertebrate tropomyosins are not found to be allergens (Liu et al. 2013; Ruethers et al. 2018).

1.2.2.4 Troponin

Troponins are a composite of three subunits named T, I, and C that are present in striated muscles. The T subunit attaches itself to tropomyosin among them. Like calmodulin and myosin light chains, troponin C shares similarities with other proteins that bind calcium ions (Ochiai and Osawa 2020). When Ca^{2+} binds to the C subunit, a dynamic structural change occurs that causes muscle contraction, while the I subunit prevents myosin from interacting with actin. Periodically, each of these subunits makes up the troponin complex, which attaches to the tropomyosin filament (Vinogradova et al. 2005). Troponins found in invertebrates are distinct from those found in vertebrates (Cao et al. 2019). The adductor smooth muscle of scallop (T, I, and C subunits) have molecular weights of about 40 kDa, 19 kDa, and 20 kDa, respectively; the T subunit's molecular weight is greater than that of mammalian troponin Ts (Nishita et al. 1997).

1.2.2.5 Paramyosin

The majority of invertebrate muscles contain a significant quantity of paramyosin. Similar to myosin rods, paramyosin dimers have coiled-coil structures and are made up of subunits that are about 100 kDa (Kajita et al. 2018). Out of the total myofibrillar protein in oysters, the adductor and top shell muscles contain about 40% of the protein, whereas the smooth muscle of scallops and the arm of octopuses contain about 30% of the protein (Ochiai et al. 1985). At the core of the thick filament of invertebrates, where myosin molecules cover the surface, is paramyosin, which forms a thick filament under physiological ionic strength (Oiwa et al. 1998). It is thought to play a role in the "catch mechanism" of bivalves, which allows them to seal their shells tightly without using any energy. Like myosin, paramyosin gels when heated become soluble at high salt concentrations (Ochiai and Osawa 2020).

1.2.3 Stroma or Connective Tissue Proteins

The remaining muscle proteins, which are insoluble in salt solutions, account for approximately 10% of the protein in elasmobranchii and 3% in teleostei (as opposed to 17% in mammals) (Sikorski et al. 1994). They are made of collagen, elastin, and connective tissue. Because fish live in an environment that does not require as much support as terrestrial animals do, their collagen content is lower in finfish than in terrestrial animals. Compared to terrestrial mammals, fish collagen is much more soluble (Hultin 1984). The texture of fish fillets may be softer than that of terrestrial animal fillets due to lower collagen content in fish (Sato et al. 1986; Zhong et al. 2023). In animal connective tissue and extracellular matrix, collagen serves as the primary structural protein. Collagen protein is found in large quantities in mammals

and is primarily found in the extracellular matrix of fibrous connective tissues, including the skin and tendons (Sorushanova et al. 2019). Comprising three polypeptide alpha-chains, collagen is a trimeric molecule that assembles into intricately arranged three-dimensional structures that can withstand mechanical stress and facilitate cellular growth. In order for connective tissues to be structurally stable and resistant to mechanical stress, fibrils made of collagen types I, II, III, V, and XI are required. The skin and tendons contain the majority of type I collagen, which is the most prevalent type (Nicol et al. 2019; Geahchan et al. 2022).

1.3 Fish Protein-Based Products

Fish consumption is common in daily human meals. Studies have shown that dietary supplements containing fish are beneficial for treating a variety of inflammatory-related illnesses, including hyperlipidemia, ulcerative colitis, and cardiovascular disease (Wang et al. 2006; Siscovick et al. 2017). It is well known that fish proteins have significant nutritional value and health benefits. These characteristics imply that fish protein is a valuable pure material that can be used to prepare a variety of food products (Borda et al. 2017). Typically, only 20–50% of the processed seafood is recovered as edible portions; the remaining 80–50% is thrown away as "nonedible" leftovers (approximately 20 MT globally). Along with being an excellent source of other beneficial ingredients, these seafood by-products are also rich in protein and peptides, minerals, vitamins, collagen and gelatin, oil and lipids, pigments, chitin, enzymes, and flavors (Pal and Suresh 2016). The myofibrillar protein quality is thought to be the utmost vital component of the extraction process since it influences the physical stability of functional foods that have been formulated as well as the interactions between proteins (Chen et al. 2017).

1.3.1 Fish Protein Isolate (FPI)

A unique supply of animal protein is fish protein isolate (FPI) that can be utilized to enhance animal feed and create food products with additional value. One technique that is used to recover fish proteins is the pH-shift or acid-alkaline solubilization process (Batista 1999). FPI can be added to food formulations as an ingredient or additive to improve functional qualities or increase nutritional values. Using FPI, superfoods and functional foods with palatable nutritional and sensory attributes can be created (Shabanpour and Etemadian 2016). In addition to increasing the amount of ω-3 PUFAs in the diet, it can also be used as a vehicle for fortification with a number of beneficial ingredients (Ananey-Obiri and Tahergorabi 2018). There have also been reports of using FPI as an emulsifier (Nolsøe and Undeland 2009; Shaviklo and Etemadian 2019).

1.3.2 Fish Protein Hydrolysate (FPH)

The process of turning seafood into fish protein hydrolysates (FPH) is regarded as one of the best and most efficient ways to extract important proteins or peptides from seafood. FPH can be created by employing chemicals or enzymes to hydrolyze fish muscle or other body parts (Chen et al. 1998). With a compound annual growth rate of more than 5%, the market for fish protein hydrolysates is predicted to reach USD 558 million by 2025. This is because there is an increasing need for protein-based food formulations, supplements, infant foods, aquafeeds, and fertilizers because of their higher absorption or digestion (Nikoo et al. 2022, 2023). Peptides that result from the hydrolysis process typically consist of two to twenty amino acids. The sequences of the amino acids and their various functional effects were intimately related. Antimicrobial, antihypertensive, and antioxidant properties are known to exist in several fish peptides (Chen et al. 2022). Enzymatic hydrolysis-derived marine protein hydrolysates demonstrated antioxidant activity against pro-oxidative metal ions and free radicals. Therefore, they might be employed as substitute antioxidants in food and the body to combat ageing caused by free radicals (Liu et al. 2022). Seafood lipid and protein oxidation can be inhibited by marine protein hydrolysates and peptides, suggesting that they have anti-freezing and antioxidant properties (Tang et al. 2023; Nikoo et al. 2023).

1.3.3 Fish Protein Concentrate (FPC)

Fish protein concentrate (FPC) is any steady protein produced for human consumption and has a higher protein concentration than the original fish. Fish is processed to make fish protein concentrate (FPC) by draining the oil, screening, settling the bones, and finally drying. In comparison to fish meal, FPC has a lower ash content and a higher protein content (about 80%). If the water and oil have been removed, fish hydrolysate and FPC are similar. FPC has a more homogeneous texture and color and a smaller particle size than FM. Owing to the higher production costs and associated processing expenses, FPC is only manufactured for human consumption and a few specific uses, mostly as milk substitutes. The unpleasant fishy taste is significantly reduced in FPC due to its low oil content (Saleh et al. 2022).

1.3.4 Fish Protein Solution (FPS)

Fish protein solution (FPS), made with an acid/alkaline assisted process, is a semi-solid protein colloid in water. Polyphosphate and NaCl are also sometimes added according to the intended application (Shaviklo et al. 2012). In the fish fillet industry, to inject into the fish fillet, FPS, which contains 0.5–1% proteins, is utilized. This could boost the fillet's microbial stability, weight, and yield (Valsdottir et al. 2006). According to Nolsøe and Undeland (2009), the implementation of FPS as a

fat blocker during the breading process can decrease the product's absorption of oil (Shaviklo and Etemadian 2019).

1.3.5 Surimi and Surimi-Based Products

Isolated from whole fish, fish parts, or bycatch, surimi is a wet and stabilized concentrate of myofibrillar protein. Initially, the fish meat is recovered through mechanical deboning. The recovered mince can be used to make a variety of foods, including coated, extruded, and surimi-based restructured products, sausages, noodles, and many more (Venugopal and Sasidharan 2022). The traditional Japanese products made with surimi are kamaboko, chikuwa, hanpan, and satsum-age. At present, fish sausage, analogue, or imitation products like crab sticks, shrimp analogues, lobster analogues, and scallop analogues are the main uses of surimi. To make surimi, the minced fish is repetitively washed in cold water to get rid of soluble ingredients like pigments, enzymes, and lipids that stick to the fish's flesh. To preserve the functionality of the proteins, surimi can be stored frozen in the presence of cryoprotectants. When surimi is exposed to warm temperatures and small amounts of salt, it gels (Venugopal et al. 1995; Venugopal and Shahidi 1996). Restructured imitation products like shrimp, crab, etc. can be made using surimi gel. The favorable functional properties of Alaska Pollock's surimi make it a conventional choice for surimi preparation. Additional species utilized in the manufacturing of surimi comprise threadfin bream, stripped mullet, sea bream, croaker, barracuda, leather jacket, lizardfish, red big eye, and cutlass fish, etc. (Venugopal and Sasidharan 2022). Surimi can be dehydrated to a protein powder in the presence of cryoprotectants such as sucrose and polyols, preventing denaturation of the proteins during drying and storage. In addition to the widely accepted restructured/analogue products made from surimi, other products that can be made with this raw material include surimi powder, paste, extrusion-cooked, dehydrated products and other ready-to-eat and ready-to-prepare value-added products (Lee 2002; Santana et al. 2012; Venugopal and Sasidharan 2022).

1.3.6 Marine Collagen

Many bioactive substances found in marine organisms have potential applications in the pharmaceutical and cosmetic industries. A substantial supply of collagen can be found in marine organisms like fish, jellyfish, sponges, and other invertebrates. These sources are superior to others because they are free of animal pathogens, have no restrictions on their religious beliefs, and have a compatible metabolism (Geahchan et al. 2022). Because marine collagen is highly accessible, soluble in water, and compatible with metabolism, it can be used as a biomaterial. Collagen is gaining popularity as an industrial ingredient in medications, food, beverages, cosmetics, tissue engineering, and health care applications because of its many benefits (Barzkar et al. 2023). The prevention and treatment of osteoporosis and

osteoarthritis have both been shown to benefit from marine collagen and its derivatives. In the field of medicine, collagen finds many uses, including drug delivery, bone and tissue regeneration, and wound healing (Song et al. 2006; Subhan et al. 2015; Geahchan et al. 2022).

1.3.7 Collagen Peptides

Collagen can be used in a variety of formulations, including scaffold-like structures, collagen fibers, collagen peptides, and collagen hydroxylates. Collagen can be hydrolyzed chemically or enzymatically to produce marine collagen peptides, which are more absorbable due to their smaller molecular weight and increased water solubility (Hu et al. 2017). Collagen hydrolysate-derived peptides have biological activities and regulatory functions in addition to their nutritional value, which can help relieve the symptoms of chronic illnesses and advance overall health. peptides with anti-osteoporotic, immuno-modulatory, chelating/absorbing metal, and hyperlipidemic properties have been demonstrated in clinical trials and drug development (Hadfi and Sarbon 2019). Numerous biological activities of hydrolyzed collagen are beneficial to industry, medicine, food, and nutrition. Hydrolysate is also thought to be beneficial in the treatment of preservatives, diabetes mellitus, gastric ulcers, skin hydration, and osteoporosis (Barzkar and Sohail 2020; Geahchan et al. 2022; Barzkar et al. 2023).

References

Abdollahi M, Wu H, Undeland I (2021) Impact of processing technology on macro and micronutrient profile of protein-enriched products from fish backbones. Food Secur 10(5):950. https://doi.org/10.3390/foods10050950

Ananey-Obiri D, Tahergorabi R (2018) Development and characterization of fish-based superfoods. Curr Top Superfoods 395:116–124

Balami S, Sharma A, Karn R (2019) Significance of nutritional value of fish for human health. Malyasian J Halal Res 2:32–34. https://doi.org/10.2478/mjhr-2019-0012

Barzkar N, Sohail M (2020) An overview on marine cellulolytic enzymes and their potential applications. Appl Microbiol Biotechnol 104:6873–6892. https://doi.org/10.1007/s00253-020-10692-y

Barzkar N, Sukhikh S, Babich O, Maran BAV, Jahromi ST (2023) Marine collagen: purification, properties and application. Front Mar Sci 10:1245077. https://doi.org/10.3389/fmars.2023.1245077

Batista I (1999) Recovery of proteins from fish waste products by alkaline extraction. Eur Food Res Technol 210:84–89. https://doi.org/10.1007/s002170050539

Begum M, Akter T, Minar MH (2012) Analysis of the proximate composition of domesticated stock of pangas (Pangasianodon hypophthalmus) in laboratory condition. J Environ Sci Nat Resour 5(1):69–74

Boland M, Kaur L, Chian FM, Astruc T (2018) Muscle proteins. In: Encyclopedia of food chemistry. Elsevier, Amsterdam, pp 164–179. https://doi.org/10.1016/b978-0-08-100596-5.21602-8

Bone Q, Hoar WS, Randall DJ (1978) Locomotor muscle. Fish Physiol 7:361–424. https://doi.org/10.1016/s1546-5098(08)60168-5

Borda D, Nicolau AI, Raspor P (2017) Trends in fish processing technologies. CRC Press, Boca Raton, p 331
Cao T, Thongam U, Jin JP (2019) Invertebrate troponin: Insights into the evolution and regulation of striated muscle contraction. Arch Biochem Biophys 666:40–45
Chen HM, Muramoto K, Yamauchi F, Fujimoto K, Nokihara K (1998) Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. J Agric Food Chem 46(1):49–53
Chen X, Tume RK, Xu X, Zhou G (2017) Solubilization of myofibrillar proteins in water or low ionic strength media: classical techniques, basic principles, and novel functionalities. Crit Rev Food Sci Nutr 57(15):3260–3280. https://doi.org/10.1080/10408398.2015.1110111
Chen J, Jayachandran M, Bai W, Xu B (2022) A critical review on the health benefits of fish consumption and its bioactive constituents. Food Chem 369:130874. https://doi.org/10.1016/j.foodchem.2021.130874
Dara PK, Geetha A, Mohanty U, Raghavankutty M, Mathew S, Nagarajarao RC, Rangasamy A (2021) Extraction and characterization of myofibrillar proteins from different meat sources: a comparative study. J Bioresour Bioprod 6(4):367–378. https://doi.org/10.1016/j.jobab.2021.04.004
FAO (2022) The state of world fisheries and aquaculture 2022. In: Towards blue transformation. FAO, Rome. https://doi.org/10.4060/cc0461en
Geahchan S, Baharlouei P, Rahman A (2022) Marine collagen: a promising biomaterial for wound healing, skin anti-aging, and bone regeneration. Mar Drugs 20(1):61. https://doi.org/10.3390/md20010061
Hadfi NH, Sarbon NM (2019) Physicochemical properties of silver catfish (Pangasius sp.) skin collagen as influenced by acetic acid concentration. Food Res 3(6):783–790. https://doi.org/10.26656/fr.2017.3(6).130
Hartman MA, Spudich JA (2012) The myosin superfamily at a glance. J Cell Sci 125:627–1632
Hu Z, Yang P, Zhou C, Li S, Hong P (2017) Marine collagen peptides from the skin of nile tilapia (oreochromis niloticus): characterization and wound healing evaluation. Mar Drugs 15(4):102
Huang MC, Lee CL, Ochiai Y, Watabe S (2019) Thermostability of tropomyosins from the fast skeletal muscles of tropical fish species. Fish Physiol Biochem 45:1189–1202
Hultin HO (1984) Postmortem biochemistry of meat and fish. J Chem Educ 61:289–298
Huss HH (1995) Quality and quality changes in fresh fish Italy (FAO Fisheries series no. 348). FAO, Rome, p 195
Kajita T, Takeda Y, Yoshida S, Yamada K, Matsumiya M, Fukushima H (2018) cDNA cloning of paramyosin from several kinds of squid mantle muscle. Adv Biosci Biotechnol 9(01):11–25
Kanoh S, Taniguchi J, Yamada T, Niwa E (2000) Effects of urea on surface hydrophobicity of requiem shark myosin. Fish Sci 66(4):801–803
Kawarazuka N, Bene C (2011) The potential role of small fish species in improving micronutrient deficiencies in developing countries: building evidence. Public Health Nutr 14(11):1927–1938
Lee CM (2002) Recent advances in surimi technology. Fish Sci 68:1523–1528. https://doi.org/10.2331/fishsci.68.sup2_1523
Liu R, Holck AL, Yang E, Liu C, Xue W (2013) Tropomyosin from tilapia (Oreochromis mossambicus) as an allergen. Clin Exp Allergy 43:365–377
Liu H, Yang Y, Liu Y, Cui L, Fu L, Li B (2022) Various bioactive peptides in collagen hydrolysate from salmo salar skin and the combined inhibitory effects on atherosclerosis in vitro and in vivo. Food Res Int 157:111281. https://doi.org/10.1016/j.foodres.2022.111281
López-Bote C (2017) Chemical and biochemical constitution of muscle. In: Lawrie's M eat science. Woodhead Publishing, Sawston, pp 99–158. https://doi.org/10.1016/B978-0-08-100694-8.00004-2
Love RM (1970) The chemical biology of fishes with a key to the chemical literature. In: The chemical biology of fishes. With a key to the chemical literature. Academic Press, New York
Lund EK (2013) Health benefits of seafood; is it just the fatty acids? Food Chem 140(3):413–420

Nicol L, Morar P, Wang Y, Henriksen K, Sun S, Karsdal M, Smith R, Nagamani SCS, Shapiro J, Lee B, Orwoll E (2019) Alterations in non-type I collagen biomarkers in osteogenesis imperfecta. Bone 120:70–74. https://doi.org/10.1016/j.bone.2018.09.024

Nikoo M, Benjakul S, Ahmadi, Gavlighi H (2022) Protein hydrolysates derived from aquaculture and marine byproducts through autolytic hydrolysis. Compr Rev Food Sci Food Saf 21(6):4872–4899

Nikoo M, Regenstein JM, Yasemi M (2023) Protein hydrolysates from fishery processing by-products: production, characteristics, food applications, and challenges. Food Secur 12(24):4470. https://doi.org/10.3390/foods12244470

Nishita K, Ojima T, Takahashi A, Inoue A (1997) Troponin from smooth adductor muscle of Ezogiant scallop. J Biochem 121(3):419–424

Nolsøe H, Undeland I (2009) The acid and alkaline solubilization process for the isolation of muscle proteins: state of the art. Food Bioprocess Technol 2:1–27

Nowsad AKM (2007) Participatory training of trainers, a new approach applied in fish processing. Bangladesh Fisheries Research Forum, Mymensingh, p 329

Ochiai Y, Osawa H (2020) Biochemical and physicochemical characteristics of the major muscle proteins from fish and shellfish. Fish Sci 86:729–740. https://doi.org/10.1007/s12562-020-01444-y

Ochiai Y, Kariya Y, Watabe S, Hashimoto K (1985) Heat-induced tendering of turban shell (Batillus cornutus) muscle. J Food Sci 50(4):981–984

Ochiai Y, Huang MC, Fukushima H, Watabe S (2003) cDNA cloning and thermodynamic properties of tropomyosin from walleye pol lack Theragra chalcogramma fast skeletal muscle. Fish Sci 69(5):1031–1041

Oiwa K, Yamaga T, Yamada A (1998) Direct observation of a central bare zone in a native thick filament isolated from the anterior byssus retractor muscle of Mytilus edulis using fluorescent ATP analogue. J Biochem 123(4):614–618

Okagaki T, Takami M, Hosokawa K, Yano M, Higashi-Fujime S, Ooi A (2005) Biochemical properties of ordinary and dark muscle myosin from carp skeletal muscle. J Biochem 138(3):255–262

Pal GK, Suresh PV (2016) Sustainable valorisation of seafood by-products: recovery of collagen and development of collagen-based novel functional food ingredients. Innovative Food Sci Emerg Technol 37:201–215. https://doi.org/10.1016/j.ifset.2016.03.015

Pal J, Shukla BN, Maurya AK, Verma HO, Pandey G, Amitha A (2018) A review on role of fish in human nutrition with special emphasis to essential fatty acid. Int J Fish Aquat Stud 6(2):427–430

Pazos M, Méndez L, Gallardo JM, Aubourg SP (2014) Selective-targeted effect of high-pressure processing on proteins related to quality: a proteomics evidence in Atlantic mackerel (Scomber scombrus). Food Bioprocess Technol 7:2342–2353

Perry SV (2001) Vertebrate tropomyosin: distribution, properties and function. J Muscle Res Cell Motil 22(1):5–49

Pollard TD (2016) Actin and actin-binding proteins. Cold Spring Harb Perspect Biol 8(8):a018226

Ravichandran S, Kumaravel K, Florence EP (2011) Nutritive composition of some edible fin fishes. Int J Zool Res 7(3):241–251. https://doi.org/10.3923/ijzr.2011.241.251

Roos N, Islam M, Thilsted SH (2003) Small fish is an important dietary source of vitamin A and calcium in rural Bangladesh. Int J Food Sci Nutr 54(5):329–339

Ruethers T, Taki AC, Johnston EB, Nugraha R, Le TTK, Kalic T, McLean TR, Kamath SD, Lopata AL (2018) Seafood allergy: a comprehensive review of fish and shellfish allergens. Mol Immunol 100:28–57. https://doi.org/10.1016/j.molimm.2018.04.008

Ryu B, Shin KH, Kim SK (2021) Muscle protein hydrolysates and amino acid composition in fish. Mar Drugs 19(7):77. https://doi.org/10.3390/md19070377

Saleh NE, Wassef EA, Abdel- Mohsen HH (2022) Fish protein concentrate. in sustainable fish production and processing. In: Sustainable fish production and processing. Academic Press, New York, pp 259–291

Santana P, Huda N, Yang TA (2012) Technology for production of surimi powder and potential of applications. Int Food Res J 19(4):1313–1323

Sato K, Yoshinaka R, Sato M, Shimizu Y (1986) Collagen content in the muscle of fishes in association with their swimming movement and meat texture. Nippon Suisan Gakkaishi 52:1595–1600
Shabanpour B, Etemadian Y (2016) Chemical changes and shelf-life of conventional surimi and proteins recovered using pH change method from common carp (Cyprinus carpio) muscle during 5 months storage at -18°C. Iran J Fish Sci 15:311–332
Shaviklo AR, Etemadian Y (2019) Overcoming current challenges in commercial applications of fish protein isolates in food and feed systems: a review. J Food Sci Technol 56(11):4775–4784
Shaviklo AR, Thorkelsson G, Arason S (2012) Quality changes of fresh and frozen protein solutions extracted from Atlantic cod (Gadus morhua) trim as affected by salt, cryoprotectants and storage time. Turk J Fish Aquat Sci 12(1):41–51
Sikorski ZE, Pan BN, Shahidi F (1994) Seafood proteins. Chapman & Hall, New York. https://doi.org/10.1007/978-1-4615-7828-4
Sikorski ZE, Kołakowska A, Pan BS (2020) The nutritive composition of the major groups of marine food organisms. In: Seafood: resources, nutritional composition, and preservation. CRC Press, Boca Raton, pp 29–54
Simiczyjew A, Pietraszek Gremplewicz K, Mazur AJ, Nowak D (2017) Are non-muscle actin isoforms functionally equivalent? Histol Histopathol 32:1125–1139
Siscovick DS, Barringer TA, Fretts AM, Wu JH, Lichtenstein AH, Costello RB, Chris-Etherton PM, Jacobson TA, Engler MB, Alger HM, Appel LJ, Mozaffarian D (2017) Omega-3 polyunsaturated fatty acid (fish oil) supplementation and the prevention of clinical cardiovascular disease: a science advisory from the American Heart Association. Circulation 135(15):e867–e884
Somero GN (2003) Protein adaptations to temperature and pressure: complementary roles of adaptive changes in amino acid sequence and internal milieu. Comp Biochem Physiol Part B Biochem Mol Biol 136(4):577–591
Song E, Kim SY, Chun T, Byun HJ, Lee YM (2006) Collagen scaffolds derived from a marine source and their biocompatibility. Biomaterials 27(15):2951–2961
Sorushanova A, Delgado LM, Wu Z, Shologu N, Kshirsagar A, Raghunath R, Mullen AM, Bayon Y, Pandit A, Raghunath M, Zeugolis DI (2019) The collagen suprafamily: from biosynthesis to advanced biomaterial development. Adv Mater 31(1):1801651. https://doi.org/10.1002/adma.201801651
Sousa AD, Farah CS (2002) Quantitative analysis of tropomyosin linear polymerization equilibrium as a function of ionic strength. J Biol Chem 277(3):2081–2088
Subhan F, Ikram M, Shehzad A, Ghafoor A (2015) Marine collagen: an emerging player in biomedical applications. J Food Sci Technol 52:4703–4707
Sujatha K, Joice AA, Kumaar PS (2013) Total protein and lipid content in edible tissues of fishes from Kasimodu fish landing centre, Chennai, Tamil Nadu. Eur J Exp Biol 3(5):252–257
Sun XD, Holley RA (2011) Factors influencing gel formation by myofibrillar proteins in muscle foods. Compr Rev Food Sci Food Saf 10:33–51
Tacon AGJ, Metian M (2013) Fish matters: importance of aquatic foods in human nutrition and global food supply. Rev Fish Sci 21(1):22–38
Tacon AG, Lemos D, Metian M (2020) Fish for health: improved nutritional quality of cultured fish for human consumption. Rev Fish Sci Aquac 28:449–458
Tang C, Xu Y, Zhou K, Xie Y, Ma Y, Li C, Xu F, Zhou H, Xu B (2023) Mechanism behind the deterioration in gel properties of collagen gel induced by high-temperature treatments: a molecular perspective. Food Res Int 171:112985. https://doi.org/10.1016/j.foodres.2023.112985
Valsdottir T, Finnbogadottir GA, Thorarinsdottir KA, Arason S (2006) Use of fish proteins in fillets. Injection of homogenized fish mince. IFL Report, 19-6
Venugopal V (2018) Nutrients and nutraceuticals from seafood. In: Mérillon J-M, Ramawat KG (eds) Bioactive molecules in food. Springer, Cham, pp 1397–1440
Venugopal V, Sasidharan A (2022) Functional proteins through green refining of seafood side streams. Front Nutr 9:974447. https://doi.org/10.3389/fnut.2022.974447
Venugopal V, Shahidi F (1996) Structure and composition of fish muscle. Food Rev Intl 12(2):175–197

Venugopal V, Shahidi F, Lee TC (1995) Value-added products from underutilized fish species. Crit Rev Food Sci Nutr 35(5):431–435. https://doi.org/10.1080/10408399509527708

Vieira S, Francoa AR, Fernandes EM, Amorim S, Ferreira H, Pires RA, Reis RL, Martins A, Neves NM (2018) Fish sarcoplasmic proteins as a high value marine material for wound dressing applications. Colloids Surf B Biointerfaces 167:310–317. https://doi.org/10.1016/j.colsurfb.2018.04.002

Vinogradova MV, Stone DB, Malanina GG, Karatzaferi C, Cooke R, Mendelson RA, Fletterick RJ (2005) Ca^{2+} regulated structural changes in troponin. Proc Natl Acad Sci 102(14):5038–5043

Wang C, Harris WS, Chung M, Lichtenstein AH, Balk EM, Kupelnick B, Jordan H, Lau J (2006) n–3 Fatty acids from fish or fish-oil supplements, but not α-linolenic acid, benefit cardiovascular disease outcomes in primary-and secondary-prevention studies: a systematic review. Am J Clin Nutr 84(1):5–17

Wang Y, Zhou Y, Wang XX, Ma F, Xu BC, Li PJ, Cheng CG (2020) Origin of high-pressure induced changes in the properties of reduced-sodium chicken myofibrillar protein gels containing $CaCl_2$: physicochemical and molecular modification perspectives. Food Chem 319:126535. https://doi.org/10.1016/j.foodchem.2020.126535

Zhong C, Feng Y, Xu Y (2023) Production of fish analogues from plant proteins: potential strategies, challenges, and outlook. Food Secur 12(3):614. https://doi.org/10.3390/foods12030614

Fish Structural Proteins

2

Abhilash Sasidharan

Abstract

Seafood is an important dietary constituent in human diet given its nutritive value and bioavailability of the nutritional components. The primary edible portion of the seafood being the muscles require special attention as the knowledge regarding their functionality and inherent properties could be utilized for product development and by-product utilization. The properties and functions of collagen and its derivative gelatin are significant considering their versatile application potential in various fields. The properties like gel strength, biocompatibility, and thermal characteristics could be modified or fine-tuned to befit specific applications. The myofibrillar proteins also pocesses characteristic functional properties like water holding capacity, solubility, emulsifying property, foaming properties, gelling properties, and fat binding capacity signifying their application in food and various industrial applications. This chapter attempts to shed light on the basic classification, structure, and properties of seafood structural proteins. The structure and chemistry of major structural proteins such as collagen and myofibrillar proteins are discussed.

Keywords

Structural proteins · Collagen · Gelatin · Myofibrillar proteins · Functional properties

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2.1 Introduction

Different varieties of seafood are a crucial component in human diet due to their nutritional significance and digestibility. The muscles being the primary edible portion also have followed a unique evolutionary developmental path enabling different functions that regulate stance and motion. The muscles present in fish and shellfish also demonstrate various structural and attributional similarities to the ones constituting the land animals. Therefore, the structural and functional insights into the particulars of these muscles could significantly assist in understanding the varied characteristics that are fundamental in different seafood items. Based on the structural and functional properties, muscles are broadly classified as smooth and striated. Amongst the vertebrates, the striated muscles are further subcategorized as skeletal and cardiac muscles. Skeletal muscles are then further classified as fast and slow muscles (Ochiai and Ozawa 2020). The structural proteins in seafood also perform an elementary part in the functional and physical properties of the various tissue. These proteins form the key structural components of muscle, bones, skin, and connective tissue aiding the textural and total quality of products mase out of them. The knowledge regarding these proteins could help the industry to enhance food quality, food innovation as well as sustainable utilization of processing side streams. The structural proteins majorly are composed of myofibrillar proteins, collagen and gelatin, elastin, and keratin. Collagen being the primary structural protein constitutes a considerable portion of bones, connective tissue, and skin providing elasticity, strength, and structural integrity to the tissues. Recent research has been focusing on the extraction of collagen from seafood side streams like skin and bones for developing bioactive peptides and functional proteins for application in food and biomedical fields (Jafari et al. 2020). Gelatin is a derivative of collagen prepared through hydrolysis playing pivotal role in providing gelling properties to various products. Exploring new technologies for gelatin extraction from seafood side streams could help in minimizing environmental impact with emphasis on maximum utilization of the resources (Karim and Bhat 2009). Actin and myosin, the major myofibrillar proteins found in fish muscle, play a significant role in contraction of the muscle and also determines the texture and stability of the tissue. The commercial processing of seafood muscle-based products could benefit from the advanced understanding of the structure and associated interactions of these constituents by aiding in improving the quality (Tahergorabi et al. 2011). Elastin on the other hand contributes toward the elasticity and resistance of the tissue, particularly in the skin and blood vessels of the fish and is being explored for prospective applications in biomaterial science and tissue engineering (Wang et al. 2019). Keratin is found primarily in fish scales and skin. It provides safety and structural integrity giving the characteristic texture of fish scales. Recent research points in the direction of utilizing fish scale-derived keratin in biocompatible materials and wound healing (Qin et al. 2022). Awareness regarding the properties of seafood structural protein is quintessential for their application in different industries such as biomedical and food processing with a significant leverage on sustainable utilization of

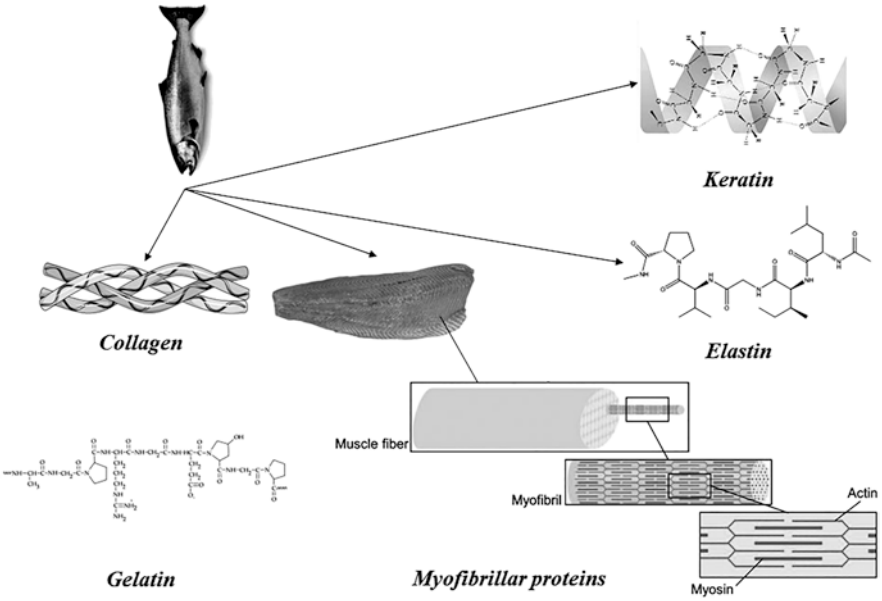

Fig. 2.1 Classification of fish structural proteins

seafood resources. Figure 2.1 represents the general classification of fish structural proteins.

2.2 Collagen

Collagen, the predominant structural protein within the body's connective tissues like skin, bones, ligaments, tendons, and cartilage (Fig. 2.2), holds a central role in numerous biomedical applications. Its versatile use spans biomaterials, including drug and gene carriers, tissue engineering, absorbable surgical sutures, osteogenic materials for bone repair, hemostatic agents, immobilization of therapeutic enzymes, and dressings for burns and wounds. Vital in the wound-healing process, collagen acts as a natural scaffold for new tissue growth, playing a pivotal role across wound-healing phases such as hemostasis, inflammation, proliferation, and remodeling.

2.2.1 Structure and Chemistry of Collagen

Collagen stands out as the most profuse proteins in the fish body, characterized by a triple helical construct comprising polypeptide α-chains (three). The collagen family encompasses 28 discovered classes to date, ranging from Type I to XXVIII (Ricard-Blum 2011). Across this superfamily, a common feature is the recurring (Gly-X-Y)n sequence, denoting the recurrence of proline (X) and hydroxyproline

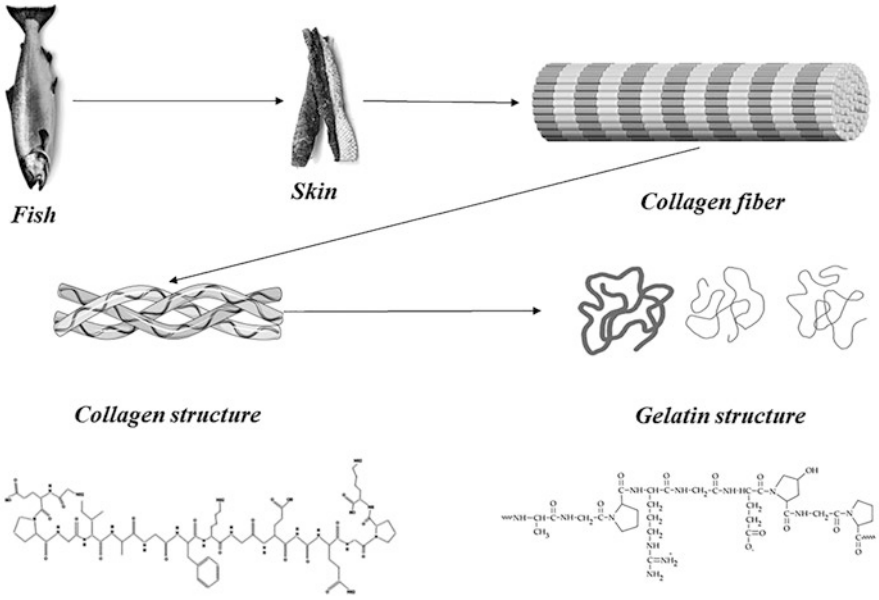

Fig. 2.2 Structure of collagen

(Y), with glycine (Gly) strictly adhering to this order. Every unit within this family contains no less than one triple-helical domain, typically sited in the extracellular matrix (Felician et al. 2018; Ricard-Blum and Ruggiero 2005; Zanaboni et al. 2000). Marine collagen, primarily identified as Type I collagen, diverges from the prevalent Type II collagen found in human collagen (80–85%) (Felician et al. 2018). Recognized for its functional properties, marine collagen has emerged as a valuable material applicable in diverse health-associated sectors, involving medicine, pharmaceuticals, food, and the cosmetics industry (Salvatore et al. 2020). Notably, its appeal extends to consumers and patients who may restrict the intake of collagen from mammalian origins (Easterbrook and Maddern 2008). The amino acid constitution and biocompatibility of aquatic collagen align closely with those of collagen from mammalian origin, dominated by <30% of glycine and 35–48% of hydroxyproline (Yamada et al. 2014). Approximately 30% of marine side streams are reported to be rich in collagen, contingent on factors such as fish species, processing methods, and by-product quality (Subhan et al. 2021). Physicochemical distinctions, including hydration behavior and thermal properties, exist between collagen extracted from cold water fish such as Atlantic or Baltic halibut, cod and warm water fish like Pacific big-eye tuna and tilapia, influencing potential applications (Gauza-Włodarczyk et al. 2017; Rose et al. 1988).

The distinctive sequence of amino acids in the collagen triple helix lends it exceptional stability, with Hyp in the X position believed to bolster this stability by fostering strong bonds with the pyrrolidine ring. The presence of the hydroxyl group in Hyp significantly influences the helix's stability, elevating both its denaturation

temperature and enthalpy. Furthermore, an arrangement of water particles envelops the triple spirals, warranting their thermal solidity by directly correlating with the number of hydrogen bonds and serving as a gauge for the extent of the triple helical structure (Meyer 2019). On another note, the denaturation temperature showcases contributions from both entropy and enthalpy to sustain the stability of the collagen triple helix. Inter-chain hydrogen connection fortifies the α-chains within the helix, providing moderate resistance against external molecular disruption (Paul and Bailey 2003). Hydrogen bonds are created when the amino group (NH) of a Glycine (Gly) residue forms a peptide bond with adjoining polypeptide carboxyl groups, effectively connecting the three chains (Yin et al. 2018). Post-translational modifications play a pivotal role in collagen's maturation and functionality. The hydroxylation of specific proline residues to form hydroxyproline is a critical step, occurring in the endoplasmic reticulum and facilitated by enzymes like prolyl hydroxylase (Rappu et al. 2019). This modification is essential for collagen's stability, influencing its ability to form proper triple helices and withstand mechanical stress. Cross-linking further fortifies collagen's structural integrity. This process involves the formation of covalent bonds between specific amino acids in adjacent collagen molecules, reinforcing the collagen fibrils and enhancing their resistance to degradation (Amirrah et al. 2022). Various enzymes and processes, such as lysyl oxidase, aid in the formation of the said cross-links, contributing toward the resilience and strength of tissues rich in collagen (Añazco et al. 2023). Collagen's interaction with water molecules is integral to its function. Water plays a crucial role in maintaining the collagen triple helix by occupying spaces connecting the chains and aiding the hydration and firmness of the structure (Cederlund and Aspden 2022). The arrangement and interaction of water molecules within collagen influence its mechanical properties, aiding in its flexibility and resilience. The diverse types of collagen, such as Type I, II, III, and beyond, exhibit distinct structural and functional characteristics. For instance, collagen Type I is abundant in tendons, skin, and bones, providing tensile strength, whereas Type II is prevalent in cartilage, resisting compression (Alcaide-Ruggiero et al. 2021). Each type's specific distribution across various tissues underscores their specialized roles in maintaining tissue integrity and function. Understanding collagen's chemistry is crucial not only for comprehending its structural significance but also for its involvement in various physiological processes. Collagen degradation, regulated by enzymes like collagenases and matrix metalloproteinases, is essential for tissue remodeling, wound healing, and turnover (Singh et al. 2023). Dysregulation of collagen metabolism can lead to various pathological conditions, emphasizing the importance of its precise synthesis, modification, and degradation in maintaining overall health.

2.2.2 Properties and Functions of Collagen

Various techniques for extracting collagen from marine organisms yield different amounts and exhibit distinct physiochemical properties in the extracted collagens. Among these methods, two commonly employed approaches are extraction through

acid hydrolyzed or soluble collagen (ASC) and pepsin-hydrolyzed or solubilized collagen (PSC), as evidenced in several studies (Diogo et al. 2021; Li et al. 2020; Chen et al. 2022). The ASC extraction process relies on an acid-collagen reaction, which enhances collagen extraction efficiency by disrupting cross-links within the collagen helix. This reaction also promotes increased revulsion between tropocollagen molecules as observed by Niu et al. (2016). In contrast, PSC extraction demonstrates higher concentration and lowered antigenicity in comparison to ASC. This is achieved through pepsin treatment, which effectively eliminates telopeptide zones and associated non-collagenous proteins as observed by Kim et al. (2013). Researchers have observed that combining enzymatic treatment using pepsin with acidic conditions enhances the output of isolated collagen in various studies (Niu et al. 2016; Hadfi and Sarbon 2019). Gelatin derived from structural proteins in seafood exhibits varied functional properties such as gel strength, viscosity, gelling, and melting temperatures, which depend on the collagen variety, its source, and the conditions used for extraction (Gómez-Guillén et al. 2011).

2.2.2.1 Gelling Properties

Seafood gelatin, distinct from mammalian versions, demonstrates inferior gelling properties at 4–12 °C and melting characteristics below 17 °C, making it a viable alternative for several industrial food applications (Sasidharan and Venugopal 2020). Reported gel strength for seafood gelatin ranges from 100 to 300 g bloom, while viscosity (varying from 2.5 to 13 cp) subject to the resource and analytical settings (Gómez-Guillén et al. 2011). These properties make seafood gelatin a versatile component, serving as a gelling agent, whipping agent, emulsifier, stabilizer, and film-forming agent in food applications.

2.2.2.2 Biological Properties

Peptides obtained from hydrolysates of collagen offer not just nutrition-related benefits but also possess significant bioactivities and directing roles that aid in mitigating symptoms associated with chronic diseases, promoting overall well-being. Extensive research in clinical studies and drug development has revealed a range of peptides showcasing immuno-modulatory, hyperlipidemic, anti-osteoporotic and metal chelating/absorbing, properties (Hadfi and Sarbon 2019). Additionally, peptides of smaller size (<5 kDa) obtained from hydrolyzed collagen extracted from squid demonstrate notable antioxidant and anti-inflammatory capabilities (Dai et al. 2018). Collagen's influence extends to stimulating fibroblast increase and enhancing synthesis of hyaluronic acid, factors noticeable in blood of humans at significant concentrations subsequent to the consumption of collagen hydrolysate. These multifaceted impacts highlight some of the optimistic contributions of collagen to human well-being (Wahyu and Widjanarko 2018). Collagen has also gained immense popularity as an ingredient across various industries such as pharmaceuticals, food, beverages, tissue engineering, cosmetics, and healthcare products owing to its versatile array of industrial claims (Hadfi and Sarbon 2019; Carvalho et al. 2020). Its widespread adoption in pharmacological and medical fields is attributed to its remarkable bioproperties, including low antigenicity, hemostatic activity, and

biodegradability (Mecwan et al. 2022). These exceptional attributes make collagen an invaluable component in numerous industrial sectors, fostering its integration into diverse products and applications.

2.2.2.3 Thermal Properties

At elevated temperatures, collagen molecules undergo structural unfolding. Determining the highest shift and denaturation temperatures of collagen involves determining the flow of energy calorimetry (Chuaychan et al. 2015). The occurrence of certain amino acids, like hydroxyproline (Hyp), significantly influences collagen's denaturation temperature. Hyp's hydroxyl group acts as a hydrogen donor through α-chains, contributing to higher denaturation temperatures in collagen with increased Hyp content. For instance, as observed by Chuaychan et al. (2015) collagen obtained using pepsin, possessed a higher Hyp concentration, exhibited a highest denaturation temperature (39.32 °C) and an enthalpy difference (ΔH) (0.91 J/g). Conversely, the ΔH and denaturation temperature of the collagen section separated with acid were 0.72 J/g and 38.17 °C, respectively. These observations underscore the correlation between collagen composition, thermal properties, and denaturation temperatures, emphasizing the significance of amino acid constituents in determining collagen's heat stability.

2.3 Myofibrillar Proteins

Typically, muscle proteins fall into three categories: myofibrillar, sarcoplasmic, and stroma/connective tissue proteins (Fig. 2.3). Myofibrillar proteins, which are the prolonged fibril proteins, constitute a significant portion of skeletal muscle (60–70% of sum of muscle proteins). This category primarily encompasses thick myosin and thin actin constituents and plays a crucial role in muscle contraction. Importantly, myofibrillar proteins contribute significantly to generating desired functional characteristics in meat products (Sun and Holley 2011). Myosin, a key constituent of myofibrillar proteins, maintains the 3D configuration in meat proteins and is pivotal for the gelatinating process in meat. This highly profuse and uneven molecule consists of two dense polypeptide chains and four lighter polypeptide chains. It breaks down into two proteolytic pieces such as the heavy meromyosin (HMM) and light meromyosin (LMM). LMM contains two parts of dense chains, though HMM comprises two dense chains and two lighter chains. Actin, which is the additional main module, reinforces the gel configuration of myosin within myofibrillar proteins. Actin occurs in two forms such as G-actin and F-actin. The transformation from G to F takes place in the occurrence of salt (López-Bote 2017). Conversely, sections like troponins and tropomyosin within myofibrillar proteins do not impact myosin gelation. The communication involving myosin and actin inside the acto-myosin compound echoes the meat tenderness (Wang et al. 2020). This relationship between these protein components significantly influences the textural qualities of meat products.

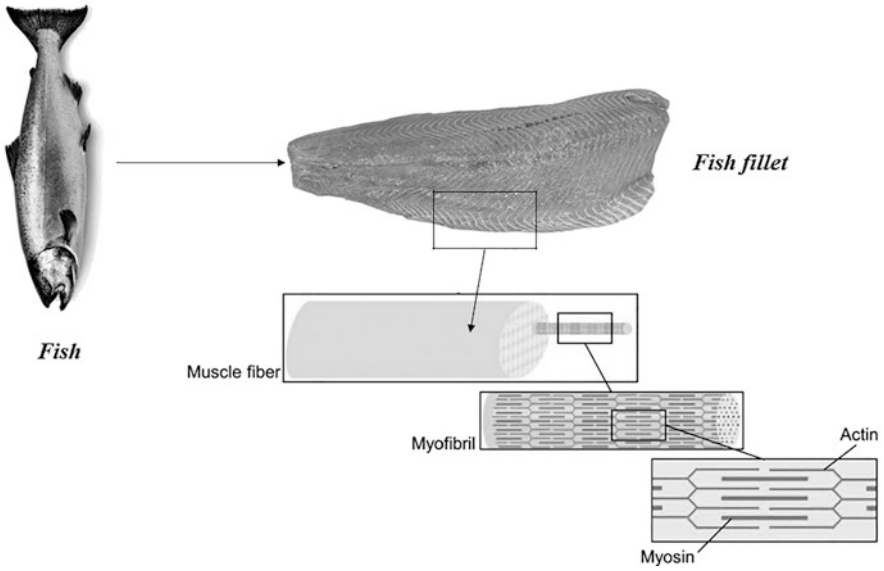

Fig. 2.3 Structure of fish myofibrillar proteins

2.3.1 Structure and Chemistry of Myofibrillar Proteins

The myofibrillar proteins, forming the most abundant protein fraction within myofibrils, consist of a limited number of proteins, possibly around a 100 as projected from 2D gel electrophoretic examination (Montowska and Pospiech 2013). These proteins are salt soluble and significantly contribute to the operational properties of both fresh and treated meat. The predominant constituents (myosin & actin), collectively make up more than 70% of the total myofibrillar protein (Vann et al. 2020). Myosin, composed of roughly 4500 amino acids and possessing a molecular weight of 500,000, exhibits a stringy arrangement comprising six sub-units consisting of two heavy chains and four light chains. These subunits are assembled into a hydrophilic helical rod tail and a hydrophobic spherical head. Conversely, actin exists in globular forms, linking together under physiological conditions to create an elongated chain (Hohmann and Dehghani 2019). In post-rigor meat, myosin and actin form cross-links as actomyosin, the predominant functional protein complex in low-salt processed meats. Alongside myosin and actin, myofibrillar proteins harbor various other proteins, containing nebulin and titin known as scaffold proteins, troponins, tropomyosin, desmin, α-actinin, C-, M-, and X-proteins, alongside several inconsequential polypeptides (Costa 2014). The specific roles of most of these subordinate proteins in muscle-based foods remain incompletely recognized. Structurally, myofibrillar proteins reside in four distinct units such as thick filaments comprising of C-protein, myosin, and M-protein, light filaments comprising of tropomyosin, actin, and the troponin composite, cytoskeletal strands such as titin and nebulin, and Z-disks such as zeugmatin, α-actinin, and desmin. These structural components

collectively contribute to the intricate organization and function of myofibrils within muscle tissues (Wang et al. 2024).

2.3.2 Properties and Functions of Myofibrillar Proteins

Fish myofibrillar proteins stand out for their distinctiveness and remarkable functional attributes. Functional properties, in this context, refer to the physicochemical traits that influence how proteins behave during food processing, ultimately impacting the quality of the end product (Zhang et al. 2021). These proteins are categorized based on solubility into myofibrillar proteins consisting of 65–75%, sarcoplasmic/enzymic proteins consisting of 20–30%, and stroma/connective tissue proteins forming around 1–3%. Among these classifications, myofibrillar proteins take the lead in deciding the functional and textural characteristics of fish meat (Fu et al. 2022). They are closely associated with the structure of the muscle. To effectively use seafood proteins in food purposes, these proteins ideally must have specific operational properties, which are intricately linked to their composition and structure (Sankar 2009). Additionally, these properties rely on how these proteins interact with other substances present within the food matrix. This relationship between the composition, structure, and interactions of fish proteins is critical for their successful utilization in various food applications. The functional attributes of seafood proteins significantly impact the quality of food products derived from these protein sources, directly influencing sensory aspects and consumer acceptance. These functionalities of seafood proteins are shaped by their overall physicochemical properties during processing, consumption, and storage. The specific sequences of peptides and amino acids within the protein structure dictate the nature and strength of these functional characteristics (Chalamaiah et al. 2012). Proteins, as complex molecules, offer a spectrum of functionalities crucial in food systems. These include their capacity to retain water, strength in gel formation, ability to emulsify, capability to create foam, and broader interactions within the food matrix involving other components. These multifaceted functionalities play a pivotal function in deciding the textural, structural, and sensory traits of food products derived from seafood proteins.

Water holding capacity (WHC): WHC of native structural proteins found in seafood plays a vital role in enhancing textural qualities such as juiciness and tenderness, contributing to the overall mouthfeel of seafood-based products (Mitchell 1998). Both WHC and drip loss (DL), indicating poor WHC, serve as markers for freshness due to their connection with water within fish muscle (Warner 2014). Unlike land-based meats, seafood generally contains a lower concentration of connective tissue proteins (Listrat et al. 2016), resulting in a comparatively lower WHC. Certain processing stages, like washing during surimi processing, have been found to improve WHC by concentrating myofibrillar protein intensities (Park 2013). Apart from inherent factors such as protein structure and amino acid composition, processing conditions like temperature, pH, and the presence of additives like NaCl significantly influence seafood protein WHC by impacting the denaturation of

structural proteins. Moreover, the methods used for protein extraction also play a substantial role in determining WHC, affecting the size and amino acid profile of peptides derived. For instance, carp proteins extracted through acid and alkaline processes exhibited WHC values of 66.7% and 62.1%, respectively, whereas water-extracted carp surimi displayed a WHC of 73.2% (Tian et al. 2017). The presence of COOH and NH2 polar groups among peptides has been identified as a significant factor influencing seafood protein WHC. Studies have shown that amino acids like glutamic and aspartic acids in rainbow trout protein hydrolysates, possessing polar side chains, notably enhance WHC (Taheri et al. 2013). Additionally, smaller peptide fragments with lower molecular weights tend to exhibit higher WHC due to their hydrophilic nature compared to larger peptide chains.

Solubility: The solubility of seafood proteins is a thermodynamic property that represents the amount of protein in a saturated solution at solid phase equilibrium, whether in a crystalline or amorphous form, under specific reference conditions (Kramer et al. 2012). This property is influenced by various external factors like pH, ionic strength of the solution, temperature, solvents, as well as intrinsic factors such as the configuration and type of amino acids present. The isoelectric characteristics of peptides significantly impact protein solubility, as the net charge of peptides is affected by the solution's pH, especially concerning weak acid or base side chain groups (Taheri et al. 2013). Peptides with lower molecular weights tend to display solubility across a wider pH range due to their higher polarity compared to larger counterparts, forming more hydrogen bonds with the aqueous solution, thus enhancing solubility. Numerous studies across different seafood varieties have demonstrated this phenomenon (Foh 2011; Betty et al. 2014). In contrast, peptides with larger molecular weights typically exhibit lower solubility because of their reduced solvent-solute affinity, resulting in fewer hydrogen bonds (Chi et al. 2014). However, extraction methods like hydrolysis can transform larger hydrophobic peptide chains into shorter, more hydrophilic peptides with carbonyl and amino side chains, consequently increasing protein solubility (Betty et al. 2014). This process alters the peptide structure, favoring greater interactions with the aqueous environment and thus enhancing solubility.

Emulsifying property: Emulsions, complex mixtures of immiscible components like oil-in-water, form under specific conditions supported by emulsifying agents. These systems, inherently unstable, find diverse applications in imparting unique textures and flavors in food and in encapsulating, protecting, and distributing functional components within food matrices (Walker et al. 2015). Stability in emulsions heavily relies on the presence of additives or emulsifying agents, functioning as surfactants, which not only maintain stability but also prevent lipid oxidation, thereby extending the emulsion's shelf life (McClements 2015a, b). Proteins, a crucial natural emulsifier group widely used in various industries, especially those sourced from seafood, exhibit amphiphilic properties. These proteins can be absorbed at the emulsion interface, effectively stabilizing lipid particles (Lam and Nickerson 2013). The emulsifying capacity of proteins is decided by the quantity and location of amino acids within the peptide chain (Damodaran 2017). Seafood proteins, owing to their surface characteristics, especially influenced by extraction

methods like hydrolysis, diminish interfacial friction between hydrophobic and hydrophilic components, thus exhibiting emulsifying properties (dos Santos et al. 2011). Reported emulsifying stability of seafood proteins varies from 0.144 to 130%, attributed to factors such as molecular size, solubility, and amino acid profile (Elavarasan et al. 2014; Chi et al. 2014; Nalinanon et al. 2011; Taheri et al. 2013). Extraction conditions like degree of hydrolysis, peptide acetylation, enzyme type, and solvent used also significantly influence this property (Nalinanon et al. 2011; Elavarasan et al. 2014; Tanuja et al. 2012; dos Santos et al. 2011). The pH of the food system notably affects emulsifying properties by altering protein surface hydrophobicity (Taheri et al. 2013). Alkaline pH tends to enhance emulsifying activity index, while acidic pH tends to diminish it (Taheri et al. 2013). Moreover, larger molecular weight peptides enhance emulsion stability, whereas lower molecular weight peptides tend to reduce it (Tanuja et al. 2012).

Foaming properties: The foaming capacity of food constituents, like proteins, holds significance in the food industry, particularly in crafting specific textured food items such as whipped cream, ice cream, and bakery goods, emphasizing volume and air incorporation (Lam et al. 2018). This property is influenced by the protein's pH sensitivity, leading to precipitation closer to its isoelectric point (Sheng-Chin Yang 2017). Additionally, the movement, reorganization, and penetration of protein particles at the air-water border dictate their foaming capabilities (Elavarasan et al. 2014). Numerous studies have reported a wide range of foaming capacities for seafood proteins, spanning from 23 to 240%, and foaming stability between 20 and 140% (Elavarasan et al. 2014; Chi et al. 2014; Taheri et al. 2013; Tanuja et al. 2012). Dispersing proteins have been observed to decrease surface tension between the air-water boundary, facilitating foam formation (Tanuja et al. 2012). The foaming capabilities of seafood proteins vary based on pH variations; at pH 4, foaming activity tends to be low, while stability is observed within the pH range of 6–10 (Taheri et al. 2013). Additionally, Betty et al. (2014) noted regarding the foaming activity of seafood peptides decreases as their molecular weight decreases. This highlights the intricate relationship between molecular characteristics and the foaming properties of seafood proteins, offering insight into their behavior under various conditions in food applications.

Gelling properties: The gelling property of seafood proteins plays a crucial role in specific food applications where texture is key. This characteristic stems from the thermal-induced partial unfolding of myosin filaments in solution, leading to irreversible accumulation and the formation of a three-dimensional structure that entraps water within the matrix. This property becomes evident after incubating seafood proteins at 0°–4 °C for around 12 h or through mild thermal processes (Sasidharan and Venugopal 2020). Disulfide bonds, particularly when treated at a pH of 11, contribute to the gelling properties of seafood proteins (Park 2013). Both the protein's inherent characteristics and the methods used for extraction influence these gelling properties significantly. The stability of the gel matrix in seafood protein gels is also influenced by protein concentrations within the matrix (Wang et al. 2015).

Fat binding capacity: The fat-binding capacity of proteins indicates their ability to absorb and retain lipid components within a matrix, closely linked to emulsifying capacity and enzyme-substrate specificity, both of which can be influenced by extraction conditions (Villamil et al. 2017). This property holds significance in the meat and confectionery industries, where it plays a role in regulating flavor characteristics (Taheri et al. 2013). Several factors contribute to the generation of a fat binding scenario in seafood proteins. The hydrophobic nature of these proteins, along with the physical properties of the oil, is crucial in establishing fat binding. Extraction methods like hydrolysis have been shown to break down peptide units, leading to specific hydrophobicity through the creation of non-polar peptide chains (He et al. 2013). The interaction between proteins and lipids involves various types of bonds, including hydrogen, electrostatic, and covalent bonds, influencing protein-lipid interactions in seafood proteins. Observations have reported the fat binding capacity of seafood proteins to range between 1.0 and 10.8 mL/g (Tanuja et al. 2012; dos Santos et al. 2011; Betty et al. 2014; Taheri et al. 2013). Notably, the fat binding capacity of seafood proteins tends to surpass that of many industrial food-grade fat binders, such as soy, milk, and casein proteins (Shaviklo and Johannsson 2007). This property showcases the potential of seafood proteins as effective components for binding and retaining lipid components within food matrices.

References

Alcaide-Ruggiero L, Molina-Hernández V, Granados MM, Domínguez JM (2021) Main and minor types of collagens in the articular cartilage: the role of collagens in repair tissue evaluation in chondral defects. Int J Mol Sci 22(24):13329. https://www.mdpi.com/1422-0067/22/24/13329

Amirrah IN, Lokanathan Y, Zulkiflee I, Wee MFMR, Motta A, Fauzi MB (2022) A comprehensive review on collagen type I development of biomaterials for tissue engineering: from biosynthesis to bioscaffold. Biomedicines 10(9):2307. https://www.mdpi.com/2227-9059/10/9/2307

Añazco C, Riedelsberger J, Vega-Montoto L, Rojas A (2023) Exploring the interplay between polyphenols and Lysyl oxidase enzymes for maintaining extracellular matrix homeostasis. Int J Mol Sci 24(13):10985. https://www.mdpi.com/1422-0067/24/13/10985

Betty M, Awuor OL, Kirwa ME, and Jackim MF (2014) Antioxidative and functional properties of Rastrineobola argentea (Dagaa) fish protein hydrolysate

Carvalho DN, López-Cebral R, Sousa RO, Alves AL, Reys LL, Silva SS, Oliveira JM, Reis RL, Silva TH (2020) Marine collagen-chitosan-fucoidan cryogels as cell-laden biocomposites envisaging tissue engineering. Biomed Mater 15(5):055030. https://doi.org/10.1088/1748-605X/ab9f04

Cederlund AA, Aspden RM (2022) Walking on water: revisiting the role of water in articular cartilage biomechanics in relation to tissue engineering and regenerative medicine. J R Soc Interface 19(193):20220364. https://doi.org/10.1098/rsif.2022.0364

Chalamaiah M, Dinesh Kumar B, Hemalatha R, Jyothirmayi T (2012) Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. Food Chem 135(4):3020–3038. https://doi.org/10.1016/j.foodchem.2012.06.100

Chen S, Hong Z, Wen H, Hong B, Lin R, Chen W, Xie Q, Le Q, Yi R, Wu H (2022) Compositional and structural characteristics of pepsin-soluble type I collagen from the scales of red drum fish, Sciaenops ocellatus. Food Hydrocoll 123:107111. https://doi.org/10.1016/j.foodhyd.2021.107111

Chi CF, Cao ZH, Wang B, Hu FY, Li ZR, Zhang B (2014) Antioxidant and functional properties of collagen hydrolysates from Spanish mackerel skin as influenced by average molecular weight. Molecules 19(8):11211–11230. https://doi.org/10.3390/molecules190811211

Chuaychan S, Benjakul S, Kishimura H (2015) Characteristics of acid-and pepsin-soluble collagens from scale of seabass (Lates calcarifer). LWT Food Sci Technol 63(1):71–76. https://doi.org/10.1016/j.lwt.2015.03.002

Costa ML (2014) Cytoskeleton and adhesion in myogenesis. ISRN Dev Biol 2014:713631. https://doi.org/10.1155/2014/713631

Dai M, Liu X, Wang N, Sun J (2018) Squid type II collagen as a novel biomaterial: isolation, characterization, immunogenicity and relieving effect on degenerative osteoarthritis via inhibiting STAT1 signaling in pro-inflammatory macrophages. Mater Sci Eng C Mater Biol Appl 89:283–294. https://doi.org/10.1016/j.msec.2018.04.021

Damodaran KLPS (ed) (2017) Fennema's food chemistry, vol 5. CRC, Boca Raton

Diogo GS, Carneiro F, Freitas-Ribeiro S, Sotelo CG, Pérez-Martín RI, Pirraco RP, Reis RL, Silva TH (2021) Prionace glauca skin collagen bioengineered constructs as a promising approach to trigger cartilage regeneration. Mater Sci Eng C 120:111587. https://doi.org/10.1016/j.msec.2020.111587

dos Santos SDA, Martins VG, Salas-Mellado M, Prentice C (2011) Evaluation of functional properties in protein hydrolysates from Bluewing Searobin (Prionotus punctatus) obtained with different microbial enzymes. Food Bioprocess Technol 4(8):1399–1406. https://doi.org/10.1007/s11947-009-0301-0

Easterbrook C, Maddern G (2008) Porcine and bovine surgical products: Jewish, Muslim, and Hindu perspectives. Arch Surg 143(4):366–370. https://doi.org/10.1001/archsurg.143.4.366

Elavarasan K, Naveen Kumar V, Shamasundar BA (2014) Antioxidant and functional properties of fish protein hydrolysates from fresh water carp (Catla catla) as influenced by the nature of enzyme. J Food Process Preserv 38(3):1207–1214. https://doi.org/10.1111/jfpp.12081

Felician FF, Xia C, Qi W, Xu H (2018) Collagen from marine biological sources and medical applications. Chem Biodivers 15(5):e1700557. https://doi.org/10.1002/cbdv.201700557

Foh MBK, Amadou MTKI, Foh BM, Wenshui X (2011) Chemical and physicochemical properties of tilapia (Oreochromis niloticus) fish protein hydrolysate and concentrate. Int J Biol Chem 5:21–36. https://scialert.net/abstract/?doi=ijbc.2011.21.36

Fu Y, Liu C, Yan X, Jiang G, Dang Q, Wang L, Liu X (2022) Physicochemical and functional properties of the muscle protein fraction of Hypomesus olidus. Food Chem X 16:100484. https://doi.org/10.1016/j.fochx.2022.100484

Gauza-Włodarczyk M, Kubisz L, Mielcarek S, Włodarczyk D (2017) Comparison of thermal properties of fish collagen and bovine collagen in the temperature range 298-670K. Mater Sci Eng C Mater Biol Appl 80:468–471. https://doi.org/10.1016/j.msec.2017.06.012

Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP (2011) Functional and bioactive properties of collagen and gelatin from alternative sources: a review. Food Hydrocoll 25(8):1813–1827. https://doi.org/10.1016/j.foodhyd.2011.02.007

Hadfi NH, Sarbon NM (2019) Physicochemical properties of silver catfish (Pangasius sp.) skin collagen as influenced by acetic acid concentration. Food Res 3:783

He S, Franco C, Zhang W (2013) Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). Food Res Int 50(1):289–297. https://doi.org/10.1016/j.foodres.2012.10.031

Hohmann T, Dehghani F (2019) The cytoskeleton—a complex interacting meshwork. Cells 8(4):362. https://www.mdpi.com/2073-4409/8/4/362

Jafari H, Lista A, Siekapen MM, Ghaffari-Bohlouli P, Nie L, Alimoradi H, Shavandi A (2020) Fish collagen: extraction, characterization, and applications for biomaterials engineering. Polymers 12(10):2230. https://www.mdpi.com/2073-4360/12/10/2230

Karim AA, Bhat R (2009) Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocoll 23(3):563–576. https://doi.org/10.1016/j.foodhyd.2008.07.002

Kim HK, Kim YH, Park HJ, Lee NH (2013) Application of ultrasonic treatment to extraction of collagen from the skins of sea bass Lateolabrax japonicus. Fish Sci 79(5):849–856. https://doi.org/10.1007/s12562-013-0648-z

Kramer RM, Shende VR, Motl N, Pace CN, Scholtz JM (2012) Toward a molecular understanding of protein solubility: increased negative surface charge correlates with increased solubility. Biophys J 102(8):1907–1915. https://doi.org/10.1016/j.bpj.2012.01.060

Lam RSH, Nickerson MT (2013) Food proteins: a review on their emulsifying properties using a structure–function approach. Food Chem 141(2):975–984. https://doi.org/10.1016/j.foodchem.2013.04.038

Lam ACY, Can Karaca A, Tyler RT, Nickerson MT (2018) Pea protein isolates: structure, extraction, and functionality. Food Rev Intl 34(2):126–147. https://doi.org/10.1080/87559129.2016.1242135

Li P-H, Lu W-C, Chan Y-J, Ko W-C, Jung C-C, Le Huynh DT, Ji Y-X (2020) Extraction and characterization of collagen from sea cucumber (Holothuria cinerascens) and its potential application in moisturizing cosmetics. Aquaculture 515:734590. https://doi.org/10.1016/j.aquaculture.2019.734590

Listrat A, Lebret B, Louveau I, Astruc T, Bonnet M, Lefaucheur L, Picard B, Bugeon J (2016) How muscle structure and composition influence meat and flesh quality. Sci World J 2016:3182746. https://doi.org/10.1155/2016/3182746

López-Bote C (2017) Chemical and biochemical constitution of muscle. In: Toldra F (ed) Lawrie's meat science, 8th edn. Woodhead Publishing, London, pp 99–158. https://doi.org/10.1016/B978-0-08-100694-8.00004-2

McClements DJ (2015a). Food emulsions

McClements DJ (2015b) Principles, practices, and techniques, vol 3. CRC Press, Boca Raton. https://doi.org/10.1201/b18868

Mecwan M, Li J, Falcone N, Ermis M, Torres E, Morales R, Hassani A, Haghniaz R, Mandal K, Sharma S, Maity S, Zehtabi F, Zamanian B, Herculano R, Akbari M, John V et al (2022) Recent advances in biopolymer-based hemostatic materials. Regen Biomater 9:rbac063. https://doi.org/10.1093/rb/rbac063

Meyer M (2019) Processing of collagen-based biomaterials and the resulting materials properties. Biomed Eng Online 18(1):24. https://doi.org/10.1186/s12938-019-0647-0

Mitchell JR (1998) Water and food macromolecules. In: Hill SE, Ledward DA, Mitchell JR (eds) Functional properties of food macromolecules. Aspen Publication, Aspen, pp 50–65

Montowska M, Pospiech E (2013) Species-specific expression of various proteins in meat tissue: proteomic analysis of raw and cooked meat and meat products made from beef, pork and selected poultry species. Food Chem 136(3–4):1461–1469. https://doi.org/10.1016/j.foodchem.2012.09.072

Nalinanon S, Benjakul S, Kishimura H, Shahidi F (2011) Functionalities and antioxidant properties of protein hydrolysates from the muscle of ornate threadfin bream treated with pepsin from skipjack tuna. Food Chem 124(4):1354–1362. https://doi.org/10.1016/j.foodchem.2010.07.089

Niu H, Wang Z, Hou H, Zhang Z, Li B (2016) Protective effect of cod (Gadus macrocephalus) skin collagen peptides on acetic acid-induced gastric ulcer in rats. J Food Sci 81(7):H1807–H1815. https://doi.org/10.1111/1750-3841.13332

Ochiai Y, Ozawa H (2020) Biochemical and physicochemical characteristics of the major muscle proteins from fish and shellfish. Fish Sci 86(5):729–740. https://doi.org/10.1007/s12562-020-01444-y

Park JW (ed) (2013) Surimi and surimi seafood, 3rd edn. CRC Press, Boca Raton. https://doi.org/10.1201/b16009

Paul RG, Bailey AJ (2003) Chemical stabilisation of collagen as a biomimetic. Scientific World J 3:138–155. https://doi.org/10.1100/tsw.2003.13

Qin D, Bi S, You X, Wang M, Cong X, Yuan C, Yu M, Cheng X, Chen X-G (2022) Development and application of fish scale wastes as versatile natural biomaterials. Chem Eng J 428:131102. https://doi.org/10.1016/j.cej.2021.131102

Rappu P, Salo AM, Myllyharju J, Heino J (2019) Role of prolyl hydroxylation in the molecular interactions of collagens. Essays Biochem 63(3):325–335. https://doi.org/10.1042/ebc20180053

Ricard-Blum S (2011) The collagen family. Cold Spring Harb Perspect Biol 3(1):a004978. https://doi.org/10.1101/cshperspect.a004978

Ricard-Blum S, Ruggiero F (2005) The collagen superfamily: from the extracellular matrix to the cell membrane. Pathol Biol 53(7):430–442. https://doi.org/10.1016/j.patbio.2004.12.024

Rose C, Kumar M, Mandal AB (1988) A study of the hydration and thermodynamics of warm-water and cold-water fish collagens. Biochem J 249(1):127–133. https://doi.org/10.1042/bj2490127

Salvatore L, Gallo N, Natali ML, Campa L, Lunetti P, Madaghiele M, Blasi FS, Corallo A, Capobianco L, Sannino A (2020) Marine collagen and its derivatives: versatile and sustainable bio-resources for healthcare. Mater Sci Eng C 113:110963. https://doi.org/10.1016/j.msec.2020.110963

Sankar TV (2009) Functional properties of fish proteins: a review. Fish Technol 46(2):87–99. https://krishi.icar.gov.in/jspui/bitstream/123456789/32212/1/Sankar%20T%20V_Functional%20Properties%20of%20Fish%20Proteins.pdf

Sasidharan A, Venugopal V (2020) Proteins and co-products from seafood processing discards: their recovery, functional properties and applications. Waste Biomass Valorization 11(11):5647–5663. https://doi.org/10.1007/s12649-019-00812-9

Shaviklo GR, Johannsson S (2007) Quality assessment of fish protein isolates using surimi standard methods

Sheng-Chin Yang REB (2017) Functional properties of eggs in foods. In: Stadelman DNJW, Newby L (eds) Egg science and technology, 5th edn. CRC, Boca Raton, p 608

Singh D, Rai V, Agrawal DK (2023) Regulation of collagen I and collagen III in tissue injury and regeneration. Cardiol Cardiovasc Med 7(1):5–16. https://doi.org/10.26502/fccm.92920302

Subhan F, Hussain Z, Tauseef I, Shehzad A, Wahid F (2021) A review on recent advances and applications of fish collagen. Crit Rev Food Sci Nutr 61(6):1027–1037. https://doi.org/10.1080/10408398.2020.1751585

Sun XD, Holley RA (2011) Factors influencing gel formation by myofibrillar proteins in muscle foods. Compr Rev Food Sci Food Saf 10(1):33–51. https://doi.org/10.1111/j.1541-4337.2010.00137.x

Tahergorabi R, Hosseini SV, Jaczynski J (2011) Seafood proteins. In: Phillips GO, Williams PA (eds) Handbook of food proteins. Woodhead Publishing, London, pp 116–149. https://doi.org/10.1533/9780857093639.116

Taheri A, Anvar SAA, Ahari H, and Fogliano V (2013) Comparison of the functional properties of protein hydrolysates from poultry by-products and rainbow trout (Onchorhynchus mykiss) viscera. http://hdl.handle.net/1834/37351

Tanuja S, Viji P, Zynudheen AA, Joshy CG (2012) Composition, functional properties and antioxidative activity of hydrolysates prepared from the frame meat of striped catfish (Pangasianodon hypophthalmus). Egypt J Biol 14:27–35

Tian Y, Wang W, Yuan C, Zhang L, Liu J, Liu J (2017) Nutritional and digestive properties of protein isolates extracted from the muscle of the common carp using pH-shift processing. J Food Process Preserv 41(1):e12847. https://doi.org/10.1111/jfpp.12847

Vann CG, Roberson PA, Osburn SC, Mumford PW, Romero MA, Fox CD, Moore JH, Haun CT, Beck DT, Moon JR, Kavazis AN, Young KC, Badisa VLD, Mwashote BM, Ibeanusi V, Singh RK, Roberts MD (2020) Skeletal muscle myofibrillar protein abundance is higher in resistance-trained men, and aging in the absence of training may have an opposite effect. Sports 8(1):7. https://www.mdpi.com/2075-4663/8/1/7

Villamil O, Váquiro H, Solanilla JF (2017) Fish viscera protein hydrolysates: production, potential applications and functional and bioactive properties. Food Chem 224:160–171. https://doi.org/10.1016/j.foodchem.2016.12.057

Wahyu YI, Widjanarko SB (2018) Extraction optimization and characterization of acid soluble collagen from milkfish scales (Chanos chanos Forskal). Carpathian J Food Sci Technol 10(1):125–135

Walker R, Decker EA, McClements DJ (2015) Development of food-grade nanoemulsions and emulsions for delivery of omega-3 fatty acids: opportunities and obstacles in the food industry. Food Funct 6(1):41–54. https://doi.org/10.1039/C4FO00723A

Wang Y, Wang R, Chang Y, Gao Y, Li Z, Xue C (2015) Preparation and thermo-reversible gelling properties of protein isolate from defatted Antarctic krill (Euphausia superba) byproducts. Food Chem 188:170–176. https://doi.org/10.1016/j.foodchem.2015.04.126

Wang R, de Kort BJ, Smits AIPM, Weiss AS (2019) Elastin in vascular grafts. In: Walpoth B, Bergmeister H, Bowlin G, Kong D, Rotmans J, Zilla P (eds) Tissue-engineered vascular grafts. Springer, Cham, pp 1–32. https://doi.org/10.1007/978-3-319-71530-8_13-1

Wang Y, Zhou Y, Wang XX, Ma F, Xu BC, Li PJ, Chen CG (2020) Origin of high-pressure induced changes in the properties of reduced-sodium chicken myofibrillar protein gels containing CaCl2: physicochemical and molecular modification perspectives. Food Chem 319:126535. https://doi.org/10.1016/j.foodchem.2020.126535

Wang Z, Qiao F, Zhang W-B, Parisi G, Du Z-Y, Zhang M-L (2024) The flesh texture of teleost fish: characteristics and interventional strategies. Rev Aquac 16(1):508–535. https://doi.org/10.1111/raq.12849

Warner R (2014) Measurement of meat quality | measurements of water-holding capacity and color: objective and subjective. In: Dikeman M, Devine C (eds) Encyclopedia of meat sciences, 2nd edn. Academic, Cambridge, pp 164–171. https://doi.org/10.1016/B978-0-12-384731-7.00210-5

Yamada S, Yamamoto K, Ikeda T, Yanagiguchi K, Hayashi Y (2014) Potency of fish collagen as a scaffold for regenerative medicine. Biomed Res Int 2014:302932. https://doi.org/10.1155/2014/302932

Yin SJ, Zhang L, Zhang L, Wan J, Song W, Jiang X, Park YD, Si YX (2018) Metabolic responses and arginine kinase expression of juvenile cuttlefish (Sepia pharaonis) under salinity stress. Int J Biol Macromol 113:881–888. https://doi.org/10.1016/j.ijbiomac.2018.03.036

Zanaboni G, Rossi A, Onana AMT, Tenni R (2000) Stability and networks of hydrogen bonds of the collagen triple helical structure: influence of pH and chaotropic nature of three anions. Matrix Biol 19(6):511–520. https://doi.org/10.1016/S0945-053X(00)00096-2

Zhang Y, Sharan S, Rinnan Å, Orlien V (2021) Survey on methods for investigating protein functionality and related molecular characteristics. Food Secur 10(11):2848. https://www.mdpi.com/2304-8158/10/11/2848

Part II

Proteomics and Bioinformatics Tools

3 Extraction, Isolation, and Characterization Techniques of Structural Proteins

Dhanya Pulikkottil Rajan

Abstract

This chapter comprehensively explores the extraction methods, isolation techniques, and characterization approaches for structural proteins. It begins by elucidating the diverse sources of protein and delineates the process of preparing crude protein extracts from these sources in the initial sub-sections. Subsequently, the focus shifts to the extraction of proteins from membranes, encompassing both extrinsic and intrinsic membrane proteins. Specifically, Section 1.2.2 delves into the extraction of fish proteins, detailing various methodologies including repeated water washing and refining, pH shift method, solvent extraction method, enzymatic hydrolysis, and autolytic hydrolysis. Furthermore, the chapter delves into protein purification methods, which include the determination of protein concentration utilizing techniques such as ultraviolet absorption, the Lowry method, the bicinchoninic acid method, the Bradford method, and Kjeldahl analysis. Additionally, it discusses fractionation methods to further enhance the understanding of structural protein isolation and characterization. Section 1.4 of this manuscript delves into the intricate process of determining protein structure, elucidating the methodologies and techniques employed in this critical aspect of protein analysis. Furthermore, Section 1.5.3 explores the influence of various external factors on protein integrity, providing insights into how factors beyond the intrinsic structure of proteins can impact their stability and functionality.

Keywords

Structural proteins · Protein isolation · Protein characterization · Fish proteins · Stability

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3.1 Introduction

Proteins are crucial biopolymers obtained from plants and animals, serving as abundant reservoirs of essential nutrients vital for body growth and development (Khan et al. 2020). In recent years, the remarkable bioactivities and functionalities of proteins have been extensively studied, gaining popularity for their contributions to health and the food processing industry (Rehman et al. 2019). The key bioactivities of proteins are linked to naturally occurring active peptides embedded in the inherent structure of the proteins (French et al. 2016).

Fish play a pivotal role as a valuable protein source, contributing significantly to nutritional, economic, cultural, and recreational aspects of human society (Lynch et al. 2016). Fish and fishery byproducts serve 60 million people as a source of diet and income (Mohanty et al. 2019). The global population surge has led to an increased demand for fish products, resulting in a noticeable expansion in fish product trade, improved preservation methods, and the industrialization of fish products (Laxe et al. 2018). Freshwater inland fish, rich in essential components like proteins, beneficial fats, and micronutrients, have the potential to enhance human health and combat malnutrition on a global scale (Nyboer et al. 2019).

3.1.1 Sources of Protein

Proteins are abundant in various sources, including cereals, plants, mammary fluids, and different parts of animals. Apart from fish flesh this includes waste materials like gut, heads, tails, skin, fins, frames, etc. (Pojić et al. 2018; Hua et al. 2019). Fish and marine products are particularly prominent as protein sources for human consumption (Petrova et al. 2018).

The protein content in fish varies by species, ranging between 8.2 and 23.9 g/100 g. Tuna has the highest protein content at 23.9%, while Bombay duck (*Harpadon nehereus*) has the lowest at 8.2%. Several fish species contain protein levels of 20% or higher (Mohanty et al. 2014).

The Indian Major Carps are significant contributors to aquaculture, containing 15–16% protein. Some minor native fishes which are not given enough significance have comparable or even higher protein content than Indian Major Carps (Mohanty et al. 2016). Examples for these are fishes belonging to genus *Anabas, Amblypharyngodon*, and *Puntius* which have a protein content higher than 16%. The Indian prawn has a protein concentration of 16.4%, which is lower than that of the tiger prawn, which has 19.4% protein. Among various fish species, *Ailia coila* has a protein concentration of 12.9%, while Bombay duck (*Harpadon nehereus*) has the lowest protein concentration at 8.2% (Mohanty et al. 2016). The concentration, quality, and type of protein can vary among different fish species and organs (Khan et al. 2020).

3.1.2 Preparation of Crude Protein Extract

The first step in any purification process involves disrupting the starting tissue to release proteins from within the cell. The method of disrupting the tissue depends on the cell type, and careful consideration must be given to the composition of the buffer used to extract the proteins.

3.1.2.1 Extraction Buffer

Typically, extraction buffers maintain an ionic strength between 0.1 and 0.2 M and a pH in the range of 7.0–8.0, which is considered compatible with the intracellular environment. Commonly used buffers include Tris or phosphate buffers. Additionally, various other reagents may be included in the buffer for specific purposes. These may include:

An antioxidant: Inside the cell, proteins exist in a highly reducing environment. However, when released into the buffer during extraction, they are exposed to a more oxidizing environment. As many proteins contain free sulfhydryl groups, derived from the amino acid cysteine, these groups can undergo oxidation, forming intermolecular and intramolecular disulfide bridges. To prevent this undesirable oxidation, reducing agents like dithiothreitol, β-mercaptoethanol, cysteine, or reduced glutathione are frequently included in the extraction buffer.

3.1.2.2 Enzyme Inhibitors

Once the cell is disrupted, the organized integrity of the cell is lost, and proteolytic enzymes, which were carefully contained and controlled within intact cells (e.g., lysosomes), are released. These enzymes may initiate the degradation of proteins within the extract, encompassing the desired protein. To alleviate undesired proteolysis, each extraction and purification stage is executed at 4 °C, and buffer receives the addition of protease inhibitors. Every inhibitor is formulated to target a particular kind of protease, such as serine proteases, thiol proteases, aspartic proteases, and metalloproteases. Instances of prevalent inhibitors consist of diisopropylphosphofluoridate, phenylmethyl sulphonylfluorid, and tosylphenylalanyl chloromethylketone for serine protease inhibition; iodoacetate and cystatin for thiol protease inhibition; pepstatin for aspartic protease inhibition; and EDTA and 1,10-phenanthroline for metalloprotease inhibition. Additionally, exopeptidase inhibitors like bestatin and amastatin might be employed.

3.1.2.3 Enzyme Substrate and Cofactors

Reduced amounts of substrate are regularly introduced into extraction buffers in the course of enzyme purification. This approach is embraced since the attachment of substrate molecules to the enzyme's active site has the potential to fortify the enzyme throughout the purification procedures. Moreover, cofactors that could be prone to loss during purification are occasionally incorporated to guarantee the preservation of enzyme activity. This facilitates the identification of enzyme activity while scrutinizing column fractions or other purification phases.

3.1.2.4 Ethylenediaminetetraacetic Acid (EDTA)

It may be included to eliminate divalent metal ions, which can otherwise interact with thiol groups in proteins, resulting in the formation of mercaptides Eq. (3.1).

$$R-SH-Me^{2+} \rightarrow R-S-Me^{+}+H^{+} \tag{3.1}$$

3.1.2.5 Polyvinylpyrrolidone

Polyvinylpyrrolidone (PVP) is often integrated into extraction buffers intended for plant tissues. This inclusion is necessary due to the substantial presence of monomeric and polymeric phenolic substances in plant tissues. Phenolic compounds tend to associate enzymes with other proteins by non-covalent bonds like hydrogen bonds, ionic, and hydrophobic bonds. This can lead to protein precipitation. Furthermore, phenolic compounds are readily oxidized by phenol oxidases in plant tissues, resulting in the formation of quinones. Quinones possess reactivity with groups of proteins, causing, aggregation, and precipitation. Counteract effects can be done by adding insoluble PVP, which will adsorb phenolic compounds. Consequently, the complex is by centrifugation. Additionally, compounds (reducing agents) introduced minimize activity phenol oxidases, thereby the formation quinones.

3.1.2.6 Sodium Azide

Buffers that are intended for long-term storage may include antifungal or antibacterial agents at mild concentrations. Sodium azide is a commonly utilized bacteriostatic substance for this purpose. It helps prevent the growth of bacteria, thus preserving the integrity of the buffer over an extended period.

3.1.3 Membrane Proteins Extraction

Special conditions are required for the extraction of proteins bound by membrane. They are not released by simple disruption procedures alone, especially glycoproteins. Membrane proteins are of two classes: extrinsic membrane proteins and intrinsic membrane proteins.

3.1.3.1 Extrinsic (Peripheral) Membrane Proteins

Extrinsic or peripheral membrane proteins are held only to the surface of the cell, typically by hydrogen and electrostatic bonds. These proteins are mainly water loving and can be relatively easily extracted. Methods for their extraction include increasing the ionic concentration of the buffer used for extraction and making changes in pH. Once extracted, these proteins can be further purified using conventional chromatographic procedures. For example, concentration can be raised to 1 M NaCl and pH can be changed to the range pH 3–5 or pH 9–12.

3.1.3.2 Intrinsic Membrane Proteins

Intrinsic membrane proteins are embedded within the membrane. Their extraction is more challenging and may involve more complex methods. These proteins are often hydrophobic and interact more strongly with the lipid bilayer. Special detergents, such as Triton X-100 or SDS (sodium dodecyl sulphate), are commonly used to solubilize and extract intrinsic membrane proteins. These detergents disrupt the lipid bilayer and help release the embedded proteins. After extraction, purification can be achieved using techniques suitable for membrane proteins, such as affinity chromatography or gel electrophoresis.

Grinding with abrasive substances: Crushing in a mortar and pestle, especially with the inclusion of sand or alumina alongside a minor quantity of buffer, proves to be an efficient approach for breaking down bacterial or plant cells. During this procedure, the abrasives actively tear apart cell walls. Nonetheless, it's crucial to emphasize that this technique is applicable primarily to manage relatively compact samples.

An alternative, larger-scale mechanical approach is the Dynomill. The Dynomill is a glass beads chamber with multiple rotating impeller discs. The disruption of cells occurs as they get captured amid beads colliding with each other. Notably, a laboratory-scale model with a capacity of 600 cm^3 can process 5 kg of bacteria per hour, making it suitable for larger-scale applications compared to the pestle-and-mortar method.

Presses: The utilization of a press, like a French Press or the Manton-Gaulin Press (a larger scale variant), offers a superb approach for breaking microbial cells. In this procedure, a cell suspension of about 50 cm^3 undergoes high pressure (10,000 FSI = lbf in-2 $\approx$ 1450 kPa) through a piston-type pump, compelling it through a small orifice. Cell rupture transpires due to shear forces when the cells traverse the narrow orifice. Furthermore, the swift pressure reduction upon exiting the orifice prompts the previously compressed cells to swiftly expand, resulting in efficient cell bursting.

Several iterations are generally needed to rupture all the cells. With precise control, there's a potential to specifically liberate proteins from the periplasmic space. Modified versions like the X-Press and Hughes Press incorporate the concept of propelling cells through the orifice as a frozen paste, frequently blended with an abrasive. In these adaptations, both the ice crystals and the abrasive play a role in breaking down the cell walls.

Enzymatic methods: Lysozyme, an enzyme isolated from hen egg whites, is capable of cleaving peptidoglycan. Treatment with lysozyme can remove the peptidoglycan cell wall from Gram-positive bacteria. In an appropriate buffer, once the cell wall undergoes digestion, the cell membrane ruptures due to the osmotic impact of the suspending buffer. For Gram-negative bacteria, treatment with lysozyme alone proves inadequate. Additional steps involving EDTA (to eliminate calcium, disrupting the outer lipopolysaccharide layer) and the incorporation of a non-ionic detergent to dissolve the cell membrane become essential. This renders the outer membrane permeable, facilitating lysozyme access to the peptidoglycan layer. Executing this procedure in an isotonic medium, where the cell membrane remains

intact, allows for the release of proteins from the periplasmic space in a selective manner.

Yeast cells can undergo disruption through enzyme action to break down the cell wall, accompanied by osmotic shock or mild physical force to breach the cell membrane. Solely relying on enzyme digestion enables the discriminative liberation of proteins from the periplasmic space. Zymolyase or lyticase are two commonly utilized enzyme preparations for yeast, both primarily featuring β 1,3-glucanase activity, coupled with a specific proteolytic function targeting the yeast cell wall. Chitinase is a common choice for disrupting filamentous fungi. Enzymatic techniques are typically applied in laboratory-scale operations, given that their utilization in extensive-scale applications is frequently constrained by expenses.

Sonication: This technique is optimal for a suspension of cultured cells or microbial cells. A sonicator probe is immersed into the cell suspension, and high-frequency sound waves (>20 kHz) are produced for 30–60. These sound waves induce cell disruption through shear force and cavitation. Cavitation denotes regions where there is alternating compression and rarefaction, rapidly interchanging. The gas bubbles in the buffer start under pressure but, as they decompress, shock waves are released, leading to cell disruption. This approach is well-suited for relatively modest volumes (50–100 cm^3). Due to the substantial heat generated, samples must be kept on ice during the procedure.

3.1.4 Extraction of Fish Proteins

Multiple methods are employed in the extraction of fish proteins from fish meat, including iterative water washing and refining, pH-shift procedures, solvent extraction, enzyme/acid hydrolysis, and heat treatment.

3.1.4.1 Repeated Water Washing and Refining

Surimi, a processed fish mince, undergoes a thorough extraction process (Fig. 3.1), where nearly all soluble proteins are removed, resulting in a composition with aqueous protein, cryoprotectants (CPA) or freeze stabilizers, and water content at approximately 16%, 75%, and 8–9%, respectively (Kobayashi and Park 2017). The elastic properties of water-insoluble proteins play a crucial role in shaping surimi meat, which can be further utilized in the production of various fish products. The initial raw materials used for myofibrillar protein extraction must be fresh and kept at low temperatures. A fishbone separator facilitates the separation of flesh from fish fillets (Muhammed et al. 2015). The leaching procedure includes mince blending in cold water, water extraction via screening, and dewatering, with the duration typically ranging from 2 to 4 min, depending on factors such as washing cycle count, unit type for washing, targeted product quality, and fish variety. A strainer/refiner is employed for the removal of residues like connective tissues and scales before final dewatering using a screw press with 0.5 mm openings, which can achieve moisture removal up to 82–85%, similar to that found in fish fillets (Muhammed et al. 2015). The leached fish flesh, combined with a cryoprotectant, is then shaped into a 10 kg block and then sealed in plastic bags or frozen in a horizontal or vertical freezer.

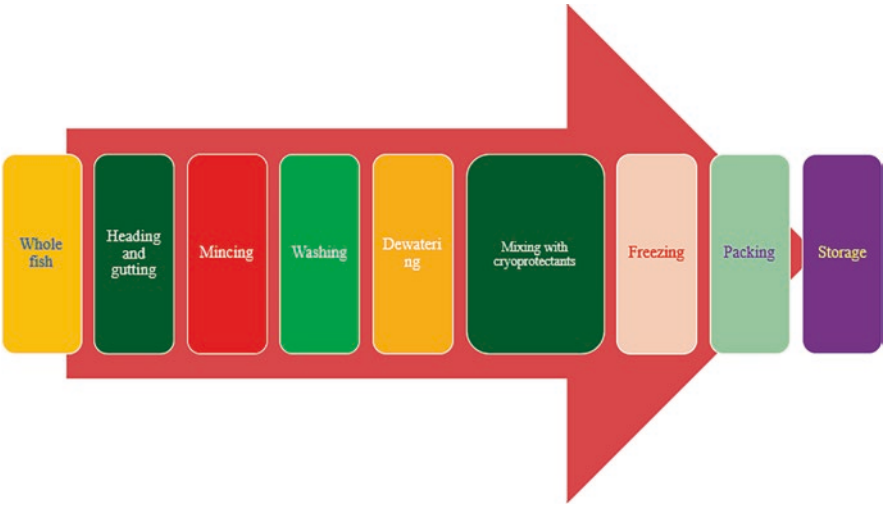


Fig. 3.1 Repeated water washing and refining technique (Khan et al. 2020)

The resulting 10 kg frozen blocks are packed in a cardboard box and stored at −20 °C (Venugopal 2005). Alkaline leaching is applied in the extraction of myofibrillar protein from muscles of dark or oily fish, like sardines and mackerels, to counteract the effects of oils and heme proteins (Hultin et al. 2005). Heme proteins like hemoglobin and myoglobin, present in dark muscles, contribute to the oxidation of fats, leading to an undesirable and rancid odor (Banerjee 2018).

3.1.4.2 pH Shift Method

Over the past few decades, numerous research studies have explored recovering protein from inexpensive fish varieties and their secondary products, successfully integrating them into the production of various nutrient-rich foods for human consumption. Initially, Hultin and Kelleher introduced a patented pH shift method, which proved highly effective in protein extraction (Hultin and Kelleher 2001). Steps in isolating fish proteins using the pH shift method given by Khan et al. (2020) is given in Fig. 3.2.

The seafood flesh is finely cut into tiny fragments and subsequently dissolved in an alkaline or acidic solution with five–ten-fold excess water volume to maintain a specific pH level (2.5 for acid or 11 for base) (Lulijwa et al. 2019). To precipitate proteins, the pH at which myofibrillar proteins are electrically neutral must be adjusted to approximately 5.2–5.5 pH (Bao et al. 2018). High-speed centrifugation is employed for the sedimentation of fish protein isolates (FPIs). Proper cryoprotectants are essential to stabilize the FPIs against freezing (Shaviklo and Etemadian 2019). FPIs can be frozen and stored similarly to surimi and fish mince for future applications.

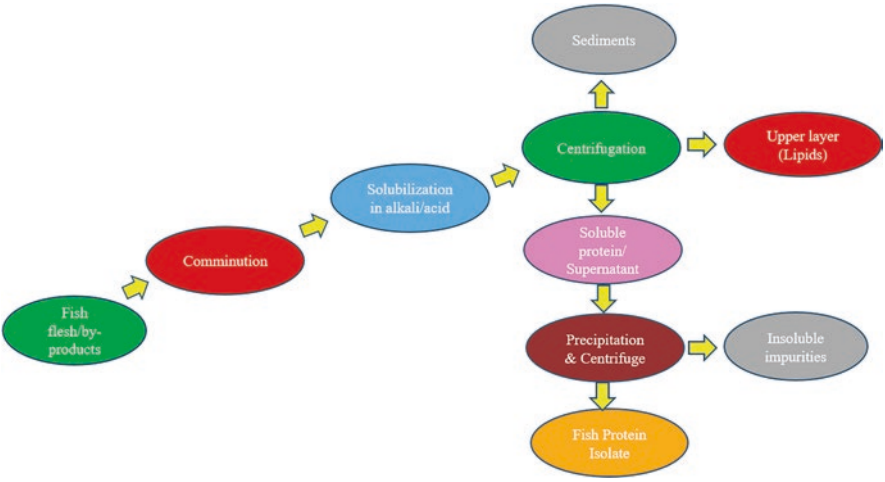


Fig. 3.2 Key steps in isolating fish proteins using the pH shift method (Khan et al. 2020)

Solvent Extraction Method

This method of extracting fish protein, primarily investigated and utilized from the 1970s to the 1980s, has recently regained significant attention from researchers (Alvarez et al. 2018). The process relies on alcohol solvents capable of removing water, fats, and components that contribute to the fishy taste of raw fish materials. Ethanol and propanol stand out as the most frequently used solvents for extracting fish protein, with ethylene dichloride being another option, contingent on the selected alcohol solvent (Shaviklo 2015). In many cases, solvents can be recycled after appropriate processing. This method generally yields three types of fish proteins. Type 1 has a fat content not exceeding 0.75% and is devoid of flavor. Type 2 may contain up to 3% fat and possesses a fishy flavor. Type 3 denotes a fundamental fish meal produced under sanitary conditions. (Sen 2005). The quality of fish proteins obtained through solvent extraction is significantly impacted by the selection of raw materials and process conditions. Lean fish, such as hake, is commonly used to produce protein, resulting in a product with a color range from light gray to yellowish-brown. Conversely, proteins extracted from oily species like anchovy and herring may exhibit a more charcoal hue, an undesirable feature limiting their use in various food applications obtained through solvent extraction (Venugopal 2005). Although the product can be stored in a hermetically sealed container for up to 6 months at 5 °C, the lack of functional characteristics remains a significant limitation for fish proteins extracted by this solvent method (Mallikage 2001).

Enzymatic Hydrolysis

Autolysis, enzymatic hydrolysis, and thermal hydrolysis are methods employed for generating hydrolysates from fish and byproducts of fish, generating bioactive compound and essential nutrients that offer numerous health benefits. Enzymatic hydrolysis has garnered significant attention from researchers due to its potential to

produce peptides, crucial ingredients in the development of commercially viable food products (Nasri et al. 2013). This technique is highly versatile and is employed using various enzymes, including protease A, trypsin, alcalase, neutrase, cryotin F, pepsin, thermolysin, bromelain, pancreatin, papain, validase, pronase, protease N, protamex, orientase, and flavorzyme. The resulting hydrolysates from enzymatic hydrolysis find applications as active components in food products (Elavarasan et al. 2014).

Proteolytic enzymes are crucial in enzymatic hydrolysis, breaking down fish proteins into soluble and insoluble fractions (Halim et al. 2016). The initial step involves homogenization at 85–90 °C for 20 min to inactivate endogenous enzymes, allowing researchers to investigate the influence of commercial enzymes on the resulting hydrolysates (Chi et al. 2015). Factors such as temperature, time, pH value, and enzyme concentration play pivotal roles in influencing the characteristics of hydrolysates produced through this method (Srichanun et al. 2014). Increasing enzyme concentration and temperature contribute to a higher count of cleaved peptide bonds until the temperature reaches the optimal level (varying for each enzyme) where denaturation occurs (Jamil et al. 2016).

Setting the pH to a defined value with sodium hydroxide or hydrochloric acid (1 N/2 N) in hydrolysis mixtures is crucial for disrupting natural protein conformations and maintaining the exact sites of enzymatic hydrolysis. Therefore, maintaining appropriate hydrolysis temperature, pH, and enzyme concentration is crucial throughout the process. Following the enzymatic hydrolysis process, enzyme activity can be halted by heating the mixture to 85–95 °C for 5–20 min, leading to enzyme inactivation (Intarasirisawat et al. 2014). The optimization of each parameter is a critical aspect of methodological decision-making (Morales and Nocedal 2002). Various methods, such as robust optimization and meta-modelling, have been proposed for their unique outcomes (Ben-Tal and Nemirovski 2002). Additionally, Utilizing Response Surface Methodology emerges as a proficient approach for optimizing and generating hydrolysate products with crucial parameters.

Autolytic Hydrolysis

Utilizing commercial enzymes allows for achieving proper pH levels through the autolysis method (Arias-Moscoso et al. 2015). Autolysis is generally based on the digestive enzymes naturally present in fish, offering a straightforward, secure, and cost-effective method. Nevertheless, its limitations arise from the reliance on particular digestive enzymes and their concentrations, posing challenges in controlling the process of hydrolysis. Autolytic techniques frequently yield a final product with suboptimal functionalities. Despite these hurdles, endogenous proteolytic enzymes (Bhaskar et al. 2008) contribute to the production of hydrolyzed products. Autolytic hydrolysis yields a viscous fluid containing small peptides and free amino acids. Fish viscera and the digestive tract are linked with digestive enzymes, including serine proteases trypsin, thiol protease pepsin, and chymotrypsin. Additionally, fish muscle cells' catheptic enzymes and lysosomal proteases participate in the proteolytic breakdown (Senphan et al. 2014). The activity of endogenous proteases

significantly influences the autolysis process, considering factors such as temperature, initial pH, and autolysis time (da Silva et al. 2017). For instance, the maximum content of total NH2-N was observed at an initial pH between 5 and 8, peaking at pH 5. The first 4 hours of autolysis showed a sharp increase in free amino nitrogen, followed by a gradual continuous increase. The free amino nitrogen content is also highly affected by temperature, with an observed increase until 40–60 °C during autolysis (Cao et al. 2009).

The initial step of the process involves blending the sample in an equivalent volume of water for 2 min, and for each enzyme, the pH of the mixture must be adjusted to the optimal activity level. A 4 M NaOH solution is constantly introduced to maintain a constant pH of the reaction mixture. Finally, the enzymatic activity is stopped by deactivating the enzyme, lowering the pH to 3.4 (Ramakrishnan et al. 2013).

3.2 Protein Purification Methods (Walker and Wilson 2010)

Purifying a specific protein from a cell and tissue homogenate, which contains 10,000–20,000 different proteins will appear like a herculean task. However, in practice, an average of only four different fractionation steps is usually required to purify a given protein. The degree of purity needed for a protein depends on the intended use for structural or functional studies. Many protein studies can be conducted on samples with 5–10% or more contamination with other proteins (Walker and Wilson 2010).

3.2.1 The Determination of Protein Concentration

Measuring the protein concentration in a solution is a regular requirement in the process of protein purification. While the most accurate method involves acid hydrolyzing a sample and performing amino acid analysis on the hydrolysate, this is time-consuming, especially for multiple samples. Fortunately, precise decimal accuracy is seldom required, and quicker methods providing reasonably accurate protein concentration assessments are acceptable. Commonly employed are colorimetric methods, which entail a reaction between a segment of the protein solution and a reagent generating a colored product. Spectrophotometric measurement of the colored product allows quantification, and calibration relates the color intensity to the protein amount. However, these methods are not absolute, as color development is influenced, at least partially, by the amino acid composition of the protein(s). The presence of prosthetic groups, such as carbohydrates, also affects colorimetric assays. Many researchers use a standard calibration curve with bovine serum albumin (BSA) due to its low cost, high purity, and easy availability.

3.2.1.1 Ultraviolet Absorption

The aromatic amino acid residues, tyrosine, and tryptophan in a protein, demonstrate an absorption maximum at a wavelength of 280 nm. As the proportions of

these aromatic amino acids vary among proteins, so do the extinction coefficients for individual proteins. However, for most proteins, the extinction coefficient typically falls within the range of 0.4–1.5. As a rough approximation for a complex protein mixture, A solution displaying an absorbance at 280 nm (A280) of 1.0, utilizing a 1 cm pathlength, correlates to a protein concentration of around 1 mg/cm. This method exhibits relative sensitivity, enabling the measurement of protein concentrations as low as 10 μg/cm. Unlike colorimetric approaches, it is non-destructive, allowing the sample in the cuvette to be utilized further. This non-destructive aspect is particularly advantageous when dealing with minimal protein quantities, emphasizing the importance of waste reduction. However, the method is vulnerable to interference from other compounds that absorb at 280 nm. Nucleic acids, with an absorbance up to 10 times that of proteins at this wavelength, fall into this category. Consequently, even a small percentage of nucleic acid presence can significantly influence the absorbance at 280 nm. However, if the absorbances *(A)* at 280 and 260 nm wavelengths are measured it is possible to apply a correction factor given in Eq. (3.2):

$$\text{Protein}\left(\text{mg cm}^{-3}\right) = 1{:}55\ \text{A}_{280} - 0.76\,\text{A}_{260} \tag{3.2}$$

This protein assay offers the significant advantage of being non-destructive and capable of continuous measurement, such as in the effluents of chromatographic columns. To achieve even greater sensitivity, peptide bonds' absorption of ultraviolet light is harnessed. The peptide bond strongly absorbs in the far ultraviolet, with a maximum at around 190 nm. Due to challenges posed by oxygen absorption and the limited output of conventional spectrophotometers at this wavelength, measurements are typically conducted at 205 or 210 nm. Most proteins exhibit an extinction coefficient of about 30 at 205 nm and approximately 20 at 210 nm for a 1 μg/cm^3 solution. Consequently, measuring at these wavelengths demonstrates a sensitivity 20–30 times higher than measuring at 280 nm, enabling the measurement of protein concentration to less than 1 μg/cm^3. However, a drawback of operating at these lower wavelengths is that numerous buffers and other components commonly employed in protein studies also exhibit strong absorption at this wavelength. As a result, it is not always practical to work at this lower wavelength. To track protein elution from columns, contemporary column chromatography systems, including high-performance liquid chromatography (HPLC) and fast protein liquid chromatography, are furnished with special detectors like in-line variable wavelength ultraviolet light detectors.

3.2.1.2 Lowry Method

The Lowry or Folin-Ciocalteau method has traditionally been a widely used technique for the determination of protein concentration. Nowadays, it is gradually being superseded by more sensitive techniques discussed below. The Lowry method exhibits reasonable sensitivity, detecting protein down to 10 μg/cm, and its sensitivity is moderately consistent across different proteins. In this method, when the Folin reagent (a mixture of sodium tungstate, molybdate, and

phosphate), along with a copper sulphate solution, is combined with a protein solution, it produces a blue-purple color that can be quantified by measuring its absorbance at 660 nm. As with many colorimetric assays, it is essential to ensure that other compounds that might interfere with the assay are not present. In the case of the Lowry method, potential interferences include Tris, zwitterionic buffers like Pipes and Hepes, and EDTA. The method relies on both the Biuret reaction, where the peptide bonds of proteins react with Cu^2+ under alkaline conditions, producing Cu + that reacts with the Folin reagent, and the Folin-Ciocalteau reaction. The latter is poorly understood but essentially involves the reduction of phosphomolybdotungstate to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic amino acids. The resulting intense blue color is, therefore, partially dependent on the tyrosine and tryptophan content of the protein sample.

Materials

The Lowry protein assay, also known as the Folin-Ciocalteu method, requires several key materials. These include Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3) solution, copper sulphate ($CuSO_4$) solution, a standard protein solution (such as bovine serum albumin, BSA), and distilled water. Additionally, a set of standard protein solutions with known concentrations is prepared to create a standard curve for quantification.

Method

To perform the Lowry protein assay, various volumes of the standard protein solution are pipetted into separate test tubes. Folin-Ciocalteu reagent is then added, and the mixture is thoroughly mixed. After a 10-min incubation, sodium carbonate solution is introduced, and the blend is allowed to incubate at ambient temperature for an additional 30 min. Following this incubation period, the absorbance of each solution is measured at approximately 750 nm using a spectrophotometer. A standard curve is constructed by plotting the absorbance versus the known protein concentrations of the standard solutions. For the samples, appropriate volumes are pipetted into test tubes, and the same procedure is followed. The absorbance readings of the samples are then converted into protein concentrations using the standard curve, providing the total protein concentration in the samples. Adhering to specific protocols and recommendations associated with the commercial kits or reagents used is crucial, as variations may exist based on the source or specific requirements of the reagents or samples.

3.2.1.3 The Bicinchoninic Acid Method

The Bicinchoninic Acid (BCA) method, akin to the Lowry method, relies on the conversion of Cu2 to Cu + under alkaline conditions. The Cu + is subsequently detected by reacting with bicinchonimic acid, resulting in a vibrant purple color with an absorbance peak at 562 nm. This method exhibits greater sensitivity than the Lowry method, capable of detecting protein concentrations as low as 0.5 μg/cm^3. A significant difference from Lowry's method is that it is generally more

tolerant of the presence of compounds that might interfere with the Lowry assay, contributing to its growing popularity.

Materials

The bicinchoninic acid (BCA) method for protein quantification involves several essential materials. These include BCA reagent, protein standards (bovine serum albumin, BSA, is commonly used), bicinchoninic acid copper (II) sulphate hydrate, sodium carbonate (Na_2CO_3), and a buffer solution. Additionally, distilled water is required for dilutions and preparation of reagent solutions.

Method

To conduct the BCA protein assay, a series of protein standards with known concentrations are prepared. Various volumes of these standards are pipetted into separate test tubes. BCA reagent is then added to each tube, and the mixture is thoroughly mixed. After an incubation period, typically at 37 °C for 30 min, sodium carbonate solution is introduced to stop the reaction. The absorbance of each solution is measured at around 562 nm using a spectrophotometer. A standard curve is constructed by plotting the absorbance against the known protein concentrations of the standards. For the samples, an appropriate volume is pipetted into a test tube, and the same procedure is followed. The absorbance readings of the samples are then compared to the standard curve to determine the protein concentrations. It's essential to follow specific guidelines and recommendations associated with the commercial kits or reagents used, as variations may exist based on the source or specific requirements of the reagents or samples.

3.2.1.4 The Bradford Method

The Bradford method relies on the binding of the dye Coomassie Brilliant Blue to proteins. At low pH, the free dye exhibits absorption maxima at 470 and 650 nm, but when bound to protein, it shows an absorption maximum at 595 nm. This method offers practical advantages, including the simplicity of reagent preparation and rapid, stable color development. Although sensitive down to 20 μg protein cm^{-3}, it is considered a relative method as the amount of dye binding seems to vary with the content of basic amino acids like arginine and lysine in the protein. This variability complicates the choice of a standard. Additionally, some proteins may not dissolve properly in the acidic reaction medium.

Materials

The Bradford protein assay, a widely used method for protein quantification, requires several key materials. These include Coomassie Brilliant Blue G-250 dye, bovine serum albumin (BSA) as a protein standard, and a protein sample or a set of protein samples to be quantified. Additionally, a Bradford reagent (a mixture of Coomassie Brilliant Blue G-250, phosphoric acid, and ethanol) is crucial for the assay. Buffered solutions, often phosphate buffer, are used to dilute the protein standards and samples. Distilled water is employed for various dilutions and preparation of the Bradford reagent.

Method

The Bradford protein assay involves the preparation of a standard curve using known concentrations of BSA. A series of BSA standard solutions are prepared, and to each, Bradford reagent is added. The solutions are mixed thoroughly and allowed to incubate for a specified time, usually around 5 min. After incubation, the absorbance of each solution is measured at approximately 595 nm using a spectrophotometer. A standard curve is generated by plotting the absorbance against the known concentrations of the BSA standards. For the protein samples, an appropriate volume is mixed with the Bradford reagent, and the procedure is repeated. The absorbance readings of the samples are then compared to the standard curve to determine their protein concentrations. As with any laboratory technique, adherence to specific guidelines and recommendations provided by the reagent or kit manufacturer is crucial for accurate and reliable results.

3.2.1.5 Kjeldahl Analysis

The Kjeldahl method is a broad chemical approach for ascertaining the nitrogen content in any compound. While it is not commonly used for analyzing purified proteins or monitoring column fractions, it is often utilized for intricate samples and microbiological samples for protein content analysis. In this method, the sample is heated with concentrated sulfuric acid in the presence of sodium sulphate (to elevate the boiling point) and a copper and/or selenium catalyst. This digestion process transforms all organic nitrogen into ammonia, which is captured as ammonium sulphate. The completion of digestion is typically indicated by the formation of a clear solution.

Ammonia is released by adding treated with sodium hydroxide and extracted through steam distillation using a Markham still. The collected ammonia is then neutralized with standard hydrochloric acid and titrated using an indicator (methyl red-methylene blue). This analysis can be carried out carried out automatically in an autokjeldahl apparatus. Alternatively, a selective ammonium ion electrode can be employed to directly measure the ammonium ion content in the digest.

While Kjeldahl analysis is a precise and reproducible method for nitrogen determination, calculating the protein content of the original sample is complicated by the variation in the nitrogen content of individual proteins and the presence of nitrogen in contaminants such as DNA. In practice, the nitrogen content of proteins is often assumed to be 16% by weight.

Materials

The Kjeldahl analysis, a commonly used technique for assessing the nitrogen content in organic samples, entails the utilization of various essential materials. This includes a Kjeldahl flask, a condenser, a distillation unit, and a receiving flask. Other essential materials encompass concentrated sulfuric acid, a catalyst such as copper sulphate or selenium, and a known quantity of the sample containing nitrogen. Alkali (commonly sodium hydroxide or potassium hydroxide) is required for the distillation step. Additionally, indicator solutions like phenolphthalein may be used to signify the endpoint of the titration.

Method

The Kjeldahl analysis method begins with the digestion of the sample in concentrated sulfuric acid. The sample is mixed with a catalyst and heated in the Kjeldahl flask. During this process, organic nitrogen is converted into ammonium sulphate. After digestion, the excess acid is neutralized by adding an alkaline solution. The liberated ammonia gas is then distilled into a receiving flask containing a known volume of an acidic solution (usually boric acid). The distilled ammonia is absorbed by this solution. The next step involves titrating the excess acid in the receiving flask with a standard alkali solution, employing an appropriate indicator to determine the endpoint of the reaction. The amount of alkali used in the titration corresponds to the ammonia generated during the digestion process. Finally, the nitrogen content in the original sample is calculated based on the volume and normality of the alkali solution used. It is important to follow standardized procedures and safety protocols while conducting Kjeldahl analysis for accurate and reproducible results.

3.2.2 Fractionation Methods

3.2.2.1 Monitoring Protein Purification

In protein purification processes, each chromatographic step generates multiple fractions with buffer content and eluted protein from the column. It is essential to ascertain the protein concentration in each tube to create an elution profile. Additionally, a method is needed to identify tubes containing the target protein for pooling and further purification steps. Should the target protein be an enzyme, measuring enzyme activity is a straightforward approach.

For proteins without easily measurable biological activity, various methods can be employed. If an antibody specific to the protein is available, dot blotting on nitrocellulose with subsequent antibody detection is an option. Immunoassays like ELISA or radioimmunoassay can also be utilized for protein detection in the fractions. In the absence of an antibody, gel electrophoresis, such as sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), can be performed, and the fraction containing the protein can be discerned by observing the presence of the protein band on the gel.

A successful fractionation step is identified by an elevation in the specific activity of the sample. The enzyme's specific activity, Eq. (3.3), indicates its total activity relative to the overall protein quantity in the preparation:

$$\text{Specific activity} = \frac{\text{total units of enzyme in fraction}}{\text{total amount of protein in fraction}} \tag{3.3}$$

Quantifying enzyme units depends on understanding fundamental kinetic principles and having an appropriate analytical procedure accessible. Enzyme activity is commonly expressed in units, and the international unit (IU) is a widely used measure. The IU is defined as the amount of enzyme that converts 1 micromole of substrate converted to the product in 1 min under specified conditions, typically at the

enzyme's optimum pH and temperature (commonly 25 or 30 °C). Additionally, the International System of Units (SI) has its unit for enzyme activity, known as the katal (kat). The SI unit represents the quantity of enzyme that converts 1 mole of substrate to the product in 1 s. The relationship between IU and kat is 1 kat = 6×10^7 IU, and 1 IU is approximately equal to 1.7×10^{-8} kat.

For a purification step to be deemed successful, the specific activity of the protein should be higher after the purification step than it was before. Expressing this enhancement is most accurately done by indicating the fold purification, Eq. (3.4).

$$\text{Fold purification} = \frac{\text{specific activity of fraction}}{\text{original specific activity}} \tag{3.4}$$

A notable rise in specific activity is evidently essential for a successful purification step. Nevertheless, another crucial factor is the yield of the step. Having an enhanced specific activity is not beneficial if you lose 95% of the protein intended for purification. Yield, Eq. (3.5), is defined as follows:

$$\text{Yield} = \frac{\text{units of enzyme in fraction}}{\text{units of enzyme in original preparation}} \tag{3.5}$$

An acceptable yield in any purification step would typically be deemed as 70% or higher.

3.2.2.2 Preliminary Purification Steps

The initial homogenate obtained from cell and tissue disruption may contain insoluble matter, including incompletely homogenized connective and vascular tissue, as well as small fragments of non-homogenized tissue. To clarify the homogenate, several steps are typically taken:

Preliminary Filtration or Centrifugation: The homogenate is often initially filtered via a dual layer of cheesecloth or subjected to low-speed centrifugation (e.g., 5000 g) to remove larger particles and debris. Fat Removal: Any fat present, which may float on the surface can be eliminated through coarse filtration using materials such as cheesecloth or glass wool. Removal of Small Particles and Organelles: Despite filtration or low-speed centrifugation, the solution may still be cloudy due to the presence of small particles and organelles. While these may be lost in subsequent purification steps, additional methods can be employed if needed. Precipitation: Materials like Celite (diatomaceous earth), Cell Debris Remover (CDR), or flocculants, like polyamines, starch, gums, or tannins, can be introduced to induce precipitation. The resultant precipitate is subsequently eliminated through centrifugation or filtration. These steps help to clarify the homogenate, making it suitable for further purification processes. The choice of methods depends on the specific characteristics of the biological material being processed and the subsequent purification steps planned.

The cell extract obtained from the homogenization process is not composed solely of proteins; it contains a variety of other molecules, including DNA, RNA, carbohydrates, lipids, and small metabolites. While small molecules are often addressed in

later purification steps, specific attention is required for macromolecules like polysaccharides and nucleic acids at this stage. Clarified extracts have to next undergo higher resolution chromatographic techniques. Determining which technique to use and the order of application is often a matter of trial and error. All purification techniques rely on harnessing the properties that distinguish proteins from one another. The diverse properties and the techniques that capitalize on these distinctions are outlined below.

Denaturation fractionation: Denaturation fractionation is a technique that takes advantage of the differences in the heat sensitivity of proteins to separate them based on their thermal stabilities. Hydrogen bonds, hydrophobic interactions, and, in certain instances, disulfide bridges typically uphold the three-dimensional (tertiary) structure of proteins. Denaturation occurs when these bonds are disrupted, causing the protein chain to unfold and rendering it insoluble.

One of the simplest methods to denature proteins in a solution is by heating. However, different proteins exhibit varying thermal stabilities, which reflects the number and strength of the bonds that contribute to their tertiary structures. The denaturation temperature for a particular protein is determined through minor-scale experiments. After this temperature is determined, it becomes feasible to selectively denature less stable undesirable proteins by heating the mixture to a temperature slightly below the critical denaturation temperature for a specific period (e.g., 15–30 min). Subsequently, the denatured, undesired proteins can be separated by centrifugation. By carefully controlling temperature and pH conditions, denaturation fractionation enables the selective removal of thermolabile proteins and facilitates the purification of the target protein of interest.

Salt Fractionation: Protein solubility is affected by the arrangement of charged polar and hydrophobic amino acids on their surfaces. Charged and polar groups are solvated by water molecules, rendering the protein soluble. In contrast, hydrophobic residues are marked by adjacent water molecules. Under specific conditions, proteins exhibit differential solubilities, and this property can be exploited for fractionation purposes, such as salt fractionation and organic solvent fractionation.

Ammonium Sulphate Precipitation: Increasing the concentration of ammonium sulphate in a protein solution leads to the solvation of salt ions by water molecules. As the salt concentration increases, water molecules solvating ions becomes fewer, and the water molecules forced into contact with hydrophobic groups on the protein's surface are the next most available. These water molecules are displaced to solvate salt molecules, revealing hydrophobic patches. Hydrophobic Interaction: With increasing ammonium sulphate concentration, hydrophobic surfaces on proteins are progressively exposed, leading to protein aggregation through hydrophobic interactions and subsequent precipitation.

Aggregation Order: Proteins aggregate based on the degree of hydrophobic residues on their surfaces, with proteins having more hydrophobic residues precipitating first, followed by those with fewer hydrophobic residues. Enrichment Procedure: While individual identical molecules do not specifically interact, this method is simple for enriching proteins of interest because many proteins precipitate over a narrow range of salt concentrations.

Organic Solvent Fractionation: Solubility in Aqueous Solutions: Organic solvent fractionation relies on variations in protein's solubility in aqueous solutions with organic solvents miscible with water like butanol, acetone, and ethanol. Dilution of Water: The addition of organic solvent dilutes water (reducing the dielectric constant), and water molecules are used to hydrate the organic solvent molecules. Solvation water is removed from charged and polar groups on protein surfaces, exposing their charged groups. Aggregation by Charge Interactions: Proteins aggregate through charge (ionic) interactions between molecules as the concentration of organic solvent increases. Order of Precipitation: Proteins precipitate in descending order based on the number of charged groups on their surfaces. Enrichment based on Charge Interactions: This method allows for the enrichment of proteins based on their surface charge characteristics.

After an initial fractionation step, higher-resolution chromatographic methods are employed for further protein purification. Various chromatographic techniques are summarized in Table 3.1, with commonly used methods detailed below.

Ion exchange chromatography: Proteins exhibit variations in the proportions of charged amino acids, such as aspartic and glutamic acids, lysine, arginine, and histidine, leading to differences in their net charge at a specific pH. This distinction is utilized in ion exchange chromatography, where the target protein is bound to a solid support material carrying charged groups of the opposite sign (ion exchange resin). Proteins with the same charge as the resin pass through the column and are discarded. Meanwhile, bound proteins, including the protein of interest, are selectively released from the column by gradually increasing the strength of salt ions in the buffer or by gradually changing the pH of the eluting buffer. In this process, ions compete with the protein for binding to the resin, with less strongly charged proteins eluting at lower salt strengths and more strongly charged proteins eluting at higher salt strengths.

Additionally, the diverse charged groups in proteins contribute to differences in their isoelectric points (pI), which is the pH at which they carry zero overall charge. This variation in pI is exploited in chromatofocusing.

Molecular exclusion chromatography: Molecular exclusion chromatography, also known as gel filtration, takes advantage of size differences among proteins. The gel filtration medium consists of beads with varying degrees of cross-linking and,

Table 3.1 Chromatographic techniques used in protein purification

Technique	Exploited Property	Capacity	Resolution
Hydrophobic interaction	Hydrophobicity	High	Medium
Ion exchange	Charge	High	Medium
Affinity	Biological function	Medium	High
Dye affinity	Structure and hydrophobicity		High
Chromatofocusing	Charge and pI	High-medium	High-medium
Covalent	Thiol groups	Medium-low	High
Metal chelate	Imidazole, thick tryptophan groups	Medium-low	High
Exclusion	Molecular size	Medium	Low

consequently, slightly different pore sizes. Separation occurs based on the varying abilities of proteins to enter, or not, certain beads, which is correlated with their size. While this method has limited resolving power, it can effectively separate large and small protein molecules, making it valuable when the protein of interest is either exceptionally large or small. Molecular exclusion chromatography is also employed for determining the relative molecular mass of a protein, as well as for concentrating or desalting protein solutions.

Affinity chromatography: Affinity chromatography exploits the specific binding interactions between certain proteins and small molecules. In this method, a ligand, which is a specific small molecule, is attached onto an insoluble support. When a blend of proteins, including the target protein, is run through the column containing the ligand-bound matrix, the target protein binds to the ligand, while other proteins flow through the column. The bound protein can then be eluted from the column by altering the pH, increasing salt strength, or passing through a high concentration of the unbound free ligand.

For example, the protein concanavalin A (con A) strongly binds to glucose. An affinity column with glucose as the ligand can be used to attach con A to the matrix, and the con A can be recovered by passing a high concentration of glucose through the column. Lectins, such as concanavalin A, are particularly useful ligands for purifying glycoproteins through affinity chromatography.

Hydrophobic Interaction chromatography: Differences in the amount of hydrophobic amino acids present on the surface of proteins can be utilized in hydrophobic interaction chromatography (HIC), a higher resolution method than salt fractionation. A common column material for HIC is phenyl-Sepharose, where phenyl groups are attached to the insoluble support Sepharose. In HIC, the protein mixture is loaded onto the column in high salt conditions to expose hydrophobic patches. Hydrophobic interactions then occur between the phenyl groups on the resin and hydrophobic regions on the proteins. Elution is achieved by applying a decreasing salt gradient, resulting in proteins emerging from the column in order of increasing hydrophobicity. For highly hydrophobic proteins that may not elute in the absence of salt, the addition of a small amount of water-miscible organic solvent, such as propanol or ethylene glycol, to the column buffer can be necessary. This solvent competes with proteins for binding to the hydrophobic matrix and helps elute any remaining proteins.

3.3 Protein Structure Determination (Walker and Wilson 2010)

Proteins are created by condensing thea among group of one amino acid or the imino group of proline with the a-carboxyl group of yet another, with the concomitant elimination of a water molecule and the creation of a peptide bond.

The gradual condensation of numerous amino acid molecules leads to the formation of an unbranched polypeptide chain. Conventionally, the N-terminal amino acid is considered the start of the chain, and the C-terminal amino acid is regarded

as the end of the chain (proteins are synthesized in this direction). Polypeptide chains contain between 20 and 2000 amino acid residues and hence have a relative molecular mass ranging between about 2000 and 200,000. Many proteins have a relative molecular mass in the range 20,000–100,000 The distinction between a large peptide and a small protein is not clear. Generally, chains of amino acids containing fewer than 50 residues are referred to as peptides, and those with more than 50 are referred to as proteins. The majority of proteins consist of several hundred amino acids. Exceptions are ribonuclease, which is an extremely small protein with only 103 amino acid residues) and many biologically active peptides contain 20 or fewer amino acids, for example, gastrin (17), somatostatin (14), oxytocin (9 amino acid residues), vasopressin (9), luteinizing hormone (10), and enkephalins (5).

The primary structure of a protein outlines the sequence of amino acid residues and is determined by the base sequence of the corresponding gene(s). Indirectly, the primary structure also defines the amino acid composition (which of the possible 20 amino acids are actually present) and content (the relative proportions of the amino acids present).

The peptide bonds linking the individual amino acid residues in a protein are both rigid and planar, with no opportunity for rotation about the carbon-nitrogen bond, as it has considerable double bond character due to the delocalization of the lone pair of electrons on the nitrogen atom; this, coupled with the tetrahedral geometry around each a-carbon atom, profoundly influences the three-dimensional arrangement which the polypeptide chain adopts.

Secondary structure defines the localized folding of a polypeptide chain due to hydrogen bonding. It includes structures such as the a-helix and B-pleated sheet. Certain of the 20 amino acids found in proteins, including proline, isoleucine, tryptophan, and asparagine, disrupt helical structures. Some proteins have up to 70% secondary structure, but others have none.

Tertiary structure defines the overall folding of a polypeptide chain by electrostatic attractions between oppositely charged ionic groups ($-NH_3$, COO^-), by weak van der Waals forces, by hydrogen bonding, hydrophobic interactions and, in some proteins, by disulphide (-S-S-) bridges formed by the oxidation of spatially adjacent sulphydryl groups (-SH) of cysteine residues. The three-dimensional folding of polypeptide chains is such that the interior consists predominantly of non-polar, hydrophobic amino acid residues such as valine, leucine, and phenylalanine. The polar, ionized, hydrophilic residues are found on the outside of the molecule, where they are compatible with the aqueous environment. However, some proteins also have hydrophobic residues on their outside and the presence of these residues is important in the processes of ammonium sulphate fractionation and hydrophobic interaction chromatography.

Quaternary structure is limited to oligomeric proteins, this involves the association of two or more polypeptide chains in oligomeric proteins, connected by electrostatic attractions, hydrogen bonding, van der Waals forces, and occasionally disulfide bridges. Disulfide bridges may exist within a specific polypeptide chain (intra-chain) or link different chains (inter-chain). An individual polypeptide chain in an oligomeric protein is termed a subunit. The subunits in a protein can be

identical or different; for instance, hemoglobin consists of two α and two β-chains, and lactate dehydrogenase has four (nearly) identical chains.

Traditionally, proteins are divided into two groups-globular and fibrous. The globular proteins are approximately spherical, are generally soluble in water, and may contain a mixture of α- helix, β-pleated sheet, and random structures. Globular proteins include enzymes, transport proteins, and immunoglobulins. Fibrous proteins are structural proteins, generally insoluble in water, consisting of long cable-like structures built entirely of either helical or sheet arrangements. Examples include hair keratin, silk fibroin, and collagen. The native state of a protein is its biologically active form. Protein structure is determined using various methods like determining relative molecular mass, mass spectroscopy, etc. The common methods are described below.

3.3.1 Methods for Determining Structure

3.3.1.1 Relative Molecular Mass

Three techniques exist for determining the relative molecular mass (Mr). or molecular weight of proteins. The first two methods are quick and easy, providing values with an accuracy of approximately ±5–10%, which is suitable for rough estimates of size. The third approach, mass spectrometry, requires specialized instruments and offers high accuracy, typically ±0.001%. Mass spectrometry is particularly valuable for detecting post-synthetic modifications of proteins.

3.3.1.2 SDS-Polyacrylamide Gel Electrophoresis

This form of electrophoresis, known as SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis), separates proteins based on their shape (size), which is related to their relative molecular masses (Mr). In this method, a set of proteins with known molecular masses, referred to as molecular weight markers, are separated on a gel alongside the protein of interest with an unknown molecular mass. The distance covered by each marker protein within the gel is measured, and a calibration curve is constructed, correlating log Mr. (relative molecular mass) against the distance traveled. The distance migrated by the protein with an unknown molecular mass is also measured, and its log Mr. and Mr. are calculated using the graph. SDS-PAGE is applicable for proteins across a broad range of molecular masses (10,000–300,000). It is a straightforward and effective technique, requiring minimal material, with detection sensitivity as low as 1 ng of protein, especially when utilizing silver staining. In practical applications, SDS-PAGE is commonly employed for determining protein Mr. values.

3.3.1.3 Molecular Exclusion Chromatography

In molecular exclusion chromatography, the elution volume of a protein from a column with an appropriate fractionation range is primarily determined by the size of the protein. This relationship follows a logarithmic pattern between the protein's relative molecular mass (Mr) and its elution volume. By calibrating the column

using a set of proteins with known Mr. values, it becomes feasible to compute the Mr. of a test protein. This method is typically performed on High-Performance Liquid Chromatography (HPLC) columns, typically measuring around 1 × 30 cm, and packed with porous silica beads. Flow rates are about 1 cm^3 min^{-1} giving a run time of about 12 min, producing sharp, well-resolved peaks. A linear calibration line is generated by plotting a graph of log Mr. versus Kd for the calibrating proteins. Kd is calculated using the following equation, Eq. (3.6):

$$K_d = \left(V_e - V_o\right) / \left(V_t - V_o\right) \tag{3.6}$$

In this equation, Vo represents the volume where molecules entirely excluded from the column material emerge (the excluded volume), Vt is the volume where small molecules capable of entering all the pores emerge (the included volume), and Ve is the volume where the marker protein elutes. This method provides values with an accuracy of ±10%.

3.3.1.4 Mass Spectrometry

Mass spectrometry, utilizing either electrospray ionization (ESI) or matrix-assisted laser desorption ionization (MALDI), allows the production of intact molecular ions for proteins, enabling accurate measurement of their masses. ESI is efficient in generating molecular ions from molecules with molecular masses up to and surpassing 100 kDa. Meanwhile, MALDI can produce ions from intact proteins. With masses up to and exceeding 200 kDa. Both methods require only low picomole quantities of protein. Mass spectrometry provides a highly precise means of determining the molecular masses of proteins and peptides, offering valuable insights into small structural changes within the protein.

3.3.1.5 Amino Acid Analysis

To determine the amino acid composition of a given protein, the protein is hydrolyzed to yield its constituent amino acids. This hydrolysis is typically achieved by heating the protein with 6 M hydrochloric acid for 14 h at 110 °C in a vacuum. However, it's important to note that this procedure destroys or chemically modifies asparagine, glutamine, and tryptophan residues. Asparagine and glutamine are converted to their corresponding acids, while tryptophan is completely destroyed and is best determined spectrophotometrically in the unhydrolyzed protein.

After hydrolysis, chromatographic separation and quantification of amino acids in the protein hydrolysate can be achieved through post-column derivatization using an appropriate reagent. In post-column derivatization approaches, the effluent from the chromatography column is combined with a reagent reacting with amino groups in amino acids, resulting in a colored or fluorescent product. The ensuing effluent is then directed through a detector (colorimeter or fluorimeter), and the degree of color/fluorescence is documented on a chart recorder, presenting each amino acid as an individual peak.

Originally, amino acid analyzers, dedicated to examining amino acids in mixtures using this method, relied on ion-exchange chromatography on a sulphated

polystyrene column with ninhydrin as the color reagent. Ninhydrin exhibited sensitivity to approximately 50–100 pmol of the amino acid. Subsequently, α-phthalaldehyde and fluorescamine emerged as favored reagents, producing fluorescent products and enabling the detection of as little as 10 pmol of an amino acid through fluorimetry.

In recent years, precolumn derivatization of amino acids, followed by separation using reversed-phase high-performance liquid chromatography (HPLC), has gained popularity and generally superseded the original ion exchange method for quantifying amino acids in a protein hydrolysate. This approach involves treating the amino acid hydrolysate with a molecule that reacts with amino groups, is hydrophobic for reversed-phase HPLC separation, and is easily detected by ultraviolet absorbance or fluorescence. Reagents like o-phthalaldehyde, 6-aminoquinolyl N-hydroxysuccinimidyl carbamate (AQC), and phenylisothiocyanate are commonly used for precolumn derivatization. This method allows for faster analysis times (as little as 20 min) and increased sensitivity, detecting amino acids down to 1 pmol or less.

3.3.2 Determination of Primary Structure

Protein Identification: Obtaining the amino acid sequence of a protein, even if it's only a short segment, allows for the identification of the protein. This is crucial in understanding the biological functions associated with the protein. The identified protein can be linked to specific cellular processes, pathways, or diseases.

Structure-Function Relationship Studies: Understanding the sequence of amino acids provides insights into the structure of the protein. Even a short sequence can reveal important structural motifs, domains, or regions. This information is valuable for studying the structure-function relationships of the protein, helping to elucidate its role in various biological processes. The advancements in protein sequencing techniques have indeed revolutionized various aspects of protein research and biotechnology.

3.3.2.1 Edman Degradation

Per Edman's groundbreaking work on the Edman degradation, published in 1950, has indeed stood the test of time as a fundamental method for sequencing amino acids in a stepwise manner from the N-terminus of a peptide or protein. The Edman degradation involves a series of three key stages:

PITC (Phenylisothiocyanate) Reaction: The N-terminal amino group of the peptide or protein reacts with PITC to form a stable phenylthiocarbamyl (PTC) derivative. This reaction is selective for the N-terminus. The reaction results in the attachment of the PTC group to the N-terminal amino acid without modifying the side chains of other amino acids.

Cleavage Reaction: After the PTC group is formed, the next step involves cleaving the N-terminal amino acid selectively from the rest of the peptide chain. The cleavage is achieved by treating the reaction product with an anhydrous acid, often

trifluoroacetic acid (TFA). This cleavage yields a phenylthiohydantoin (PTH) derivative of the N-terminal amino acid.

Separation and Detection: The final stage involves separating and identifying the PTH-amino acid derivative. Separation is typically done using chromatographic methods, and the PTH-amino acids can be detected and quantified using techniques such as HPLC (High-Performance Liquid Chromatography) or mass spectrometry. This cyclic process is repeated iteratively, allowing for the stepwise determination of the N-terminal amino acid sequence of the peptide or protein. Over the years, advancements in automation and technology have improved the efficiency and accuracy of the Edman degradation, making it an essential tool in protein sequencing methodologies.

3.3.2.2 Protein Cleavage and Peptide Generation

The Edman degradation method chiefly dictates the amino acid sequence from the N-terminus of a protein, requiring a free amino group at the N-terminus for reaction with PITC. However, a significant portion of proteins (50–70%) have their N-terminal amino group blocked (e.g., by a formyl, acetyl, or myristoyl group). In such cases, determining the N-terminal amino acid sequence becomes impractical. To address this, proteins are cleaved to produce peptides, and the amino acid sequence of these peptides can be sequenced to provide information about regions within the protein. The choice of cleavage method depends on the amino acid composition of the protein.

Chemical Cleavage Methods:

Cyanogen Bromide: Cleaves at methionine residues, producing large peptides.
N-Bromosuccinimide: Cleaves at tryptophan residues, also resulting in large peptides.

Enzymatic Cleavage Methods:

Trypsin: Cleaves C-terminal to arginine and lysine residues.
Endoproteinase Arg C: Cleaves C-terminal to arginine residues.
Endoproteinase Glu-C: Cleaves C-terminal to glutamate and some aspartate residues.

Once peptides are generated, they are often fractionated using Reverse Phase High-Performance Liquid Chromatography (RP HPLC). Despite peptides containing charged and polar groups, RP HPLC is employed because standard conditions used for separation mask the polar groups and give peptides an overall hydrophobic characteristic. Peptides are typically dissolved in 1% trifluoroacetic acid before RP HPLC. Under acidic conditions, carboxyl groups are protonated, masking the negative charge, and positively charged groups interact with trifluoroacetyl groups, masking their positive charge. The resulting appearance is of a non-charged, hydrophobic molecule, allowing separation on an RP HPLC column using a linear acetonitrile gradient to elute peptides in the order of increasing hydrophobicity.

3.3.2.3 Mass Spectrometry

Mass spectrometry (MS) analysis, historically limited to small, non-polar molecules, has undergone advancements in ionization technology, enabling the routine analysis of large, charged molecules like proteins and peptides. Techniques such as fast atom bombardment (FAB), electrospray ionization (ESI), and matrix-assisted laser desorption ionization (MALDI) have made protein and peptide analysis by MS commonplace. While the Edman degradation method still has applications, mass spectrometry is now frequently employed for amino acid sequence determination.

In MS, when peptides are fragmented, the breaks predominantly occur at the peptide bond, allowing the deduction of the amino acid sequence. Fragmentation at the peptide bond results in fragments that differ sequentially by the mass of one amino acid residue. This enables the observation of amino acid sequences and side chain modifications in proteins. Tandem mass spectrometry (MS/MS or MS2) is becoming more prevalent for acquiring sequence information. In this approach, a protein digest is subjected to separation by MS. Subsequently, a specific peptide ion is chosen and subjected to collision with argon gas, leading to the formation of fragment ions. These resulting fragments are then separated and analyzed to ascertain the sequence.

Ladder sequencing is an alternative method that integrates Edman chemistry with mass spectrometry (MS). In this approach, Edman sequencing is conducted using a blend of fluorescein isothiocyanate (FITC) and phenylisocyanate (PIC). The reaction of N-terminal amino groups with PIC serves to efficiently block them, resulting in a successive series of peptides after each round of Edman degradation. This collection of peptides, each differing by the removal of one amino acid, can be subjected to MS analysis (either Electrospray Ionization (ESI) or Matrix-Assisted Laser Desorption/Ionization (MALDI)). The molecular mass of each peptide is determined through MS, and the disparities in mass between consecutive peptides reveal the amino acid residue lost in each degradation cycle.

3.3.2.4 Detection of Disulphide Linkages

To determine the presence and number of cysteine residues joined by disulfide bridges in proteins, a common method involves the use of mass spectrometry (MS). In this approach, the native protein is cleaved using a proteolytic enzyme, such as trypsin, and a parallel experiment is conducted on proteins treated with dithiothreitol (DTT) to reduce the disulfide bridges, Eq. (3.7). MALDI spectra of the tryptic digest before and after reduction with DTT are then analyzed. Peptides linked by disulfide bridges will appear in the spectrum of the native protein but disappear after reduction with DTT, reappearing as two separate peptides of lower mass. The exact masses of these peptides, along with knowledge of the enzyme cleavage sites, allow for the identification of the peptides and determination of the presence and number of cysteine residues involved in disulfide bridges. This method provides valuable information about the structural features of proteins.

$$-\mathrm{S}-\mathrm{S}-\xrightarrow{2\mathrm{H}}\mathrm{SH}+\mathrm{HS}- \tag{3.7}$$

3.3.2.5 Hydrophobicity Profile

After determining the amino acid sequence of a protein, it becomes feasible to conduct predictive analysis on hydrophobic groups distributed along the linear sequence. This process entails constructing a hydrophobicity profile for the protein, where the average hydrophobicity per residue is plotted against the sequence number. To achieve this, a predictive algorithm is employed to calculate the mean hydrophobicity within a moving window that traverses the sequence. The outcome is a graphical representation that illustrates regions of both minimum and maximum hydrophobicity along the polypeptide chain.

In the case of membrane proteins, hydrophobicity profiles are particularly valuable for identifying potential membrane-spanning segments. For instance, analysis of a thylakoid membrane protein demonstrated seven regions in the protein sequence, each spanning 20–28 amino acid residues and primarily composed of hydrophobic residues. These regions corresponded to the seven membrane-spanning helices of the protein.

In the context of membrane proteins forming aqueous channels, the transmembrane section includes hydrophilic residues. Pores consist of amphipathic α-helices, where the polar sides line the channel and the hydrophobic sides interact with membrane lipids. Detecting these sequences involves less advanced algorithms, as they may not be readily revealed by simple hydrophobicity analysis.

3.3.2.6 Glycoproteins

Glycoproteins are formed through the covalent attachment of carbohydrate chains (glycans) with varying structures, including linear and branched configurations, to specific sites on the polypeptide backbone of a protein. These post-translational modifications take place with the involvement of enzymes in the cytoplasm within the endoplasmic reticulum and Golgi apparatus. The amount of attached polysaccharide can range widely, from a few percent to over 60% by weight. Glycoproteins are frequently present in serum and cell membranes.

The carbohydrate moiety of glycoproteins serves various crucial roles, such as stabilizing protein structure, protecting proteins from degradation by proteases, regulating protein half-life in the bloodstream, contributing to the preservation of the structure and integrity of tissues, participating in cellular adhesion and interactions between cells, and serving as a key factor in receptor-ligand interactions. The primary categories of protein glycoconjugates include:

- N-linked
- O-linked
- glycosylphosphatidylinositol (GPI) linked

N-linked glycans are always linked to an asparagine residue side chain at a consensus sequence Asn-X-Ser/Thr where X is any amino acid except proline. O-linked glycosylation occurs where carbohydrate is attached to the hydroxyl group of a serine or threonine residue. However, there is no consensus sequence similar to that found for N-linked oligosaccharides. Glycosylphosphatidylinositol (GPI)

membrane anchors represent a more recently discovered modification of proteins. These are complex glycophospholipids covalently attached to various plasma membrane proteins expressed externally. The primary role of GPI anchors is to establish a stable association between the protein and the lipid bilayer of the membrane.

Understanding the structure of O- and N-linked oligosaccharides is of significant interest because glycosylation can impact both the half-life and function of a protein. This is particularly crucial when producing therapeutic glycoproteins using recombinant methods, as it is essential to ensure the correct carbohydrate structure is generated. It's important to note that prokaryotic cells do not naturally produce glycoproteins, so genes for glycoproteins must be expressed in eukaryotic cells.

The glycosylation of proteins is a complex process with variations in glycosylation sites, the type of amino acid-carbohydrate bond, composition of sugar chains, and specific carbohydrate sequences and linkages. There are eight commonly found monosaccharide units in mammalian glycoproteins, including D-xylose (Xyl), D-mannose (Man), 1-fucose (Fuc), N-acetyl neuraminic acid (NeuNAc), D-galactose (Gal), N-acetylglucosamine (GlcNac), N-acetylgalactosamine (GalNAc), and N glycolyl neuraminic acid (NeuGc). Heterogeneity in glycoprotein populations can result in diverse carbohydrate structures (glycoforms), including increased branching, reduced chain length, and additional carbohydrate units.

Determining the glycosylation status of a molecule involves various steps. Initially, it is essential to confirm whether a protein is a glycoprotein. Glycoprotein bands in gels can be stained using methods like Alcian Blue or periodic acid-Schiff reagent (PAS). Alternatively, lectins, specific carbohydrate-binding proteins, can be used for higher sensitivity and information. Enzyme-linked lectins are employed to identify glycoproteins and determine the specific sugar residues present.

Once the presence of a glycoprotein is confirmed, further procedures are typically carried out to characterize its glycosylation status.

- Identification of the composition and quantity of individual monosaccharides involves the hydrolysis of the sample in methanolic HCl at 80 °C for 18 h. Subsequently, the liberated monosaccharides can be separated and measured using gas chromatography.
- Protease digestion is employed to release glycopeptides. A suitable protease is selected to cleave the glycoprotein into peptides and glycopeptides, ideally consisting of 5–15 amino acid residues. The resulting glycopeptides are then fractionated via HPLC, and the purified ones undergo N-terminal sequence analysis to identify the glycosylation site.
- Oligosaccharide profiling is conducted by releasing oligosaccharide chains from the polypeptide backbone, either chemically (e.g., hydrazinolysis for N-linked oligosaccharides) or enzymatically using peptide N glucosidase F (PNGase F) for cleaving sugars at the asparagine link, or endo-α-N acetylgalactosaminidase (O-glycanase) for O-linked glycans. The liberated oligosaccharides are then separated either by HPLC or high-performance anion exchange chromatography (HPAEC).

- Structural analysis of each purified oligosaccharide necessitates determining its composition, sequence, and the nature of linkages. This entails employing a combination of techniques like FAB-MS analysis, gas chromatography-MS, lectin analysis subsequent to partial sugar release, and nuclear magnetic resonance (NMR) analysis.

3.3.3 Determination of Tertiary Structure

X-ray crystallography stands out as the most widely employed technique for elucidating the three-dimensional structure of proteins. The process involves several key steps, outlined below in a concise and idealized manner.

Protein crystallization begins with the formation of a crystal, defined as a three-dimensional lattice of molecules. To maximize the chances of successful crystallization, efforts are made to create a homogeneous protein preparation, as impurities can hinder the process. The empirical nature of protein crystallization arises from a limited understanding of the underlying physical processes. The strategy involves manipulating various physical parameters affecting protein solubility - such as temperature, pH, ionic strength, and the occurrence of a precipitating agent - to induce a state of supersaturation.

This method often requires extensive trial and error to identify optimal conditions for a specific protein. Initial systematic screenings aim to detect conditions conducive to crystallinity, followed by refining these conditions for optimal crystal growth. Nucleation sites, crucial for crystal formation, result from chance collisions of molecules, leading to molecular aggregates. Achieving saturated solutions, where the likelihood of these aggregates forming is higher, typically demands tens of milligrams of proteins.

Genetic engineering advancements have notably simplified the challenge of obtaining sufficient protein quantities for crystallization. Cloned genes enable the overproduction of most proteins, allowing researchers to generate the necessary amounts almost on demand. These advances have led to the development of several successful protein crystallization methods.

3.3.3.1 Dialysis

The method of dialysis is employed to attain a state of supersaturation in the protein solution. This is accomplished by exposing the protein solution to dialysis against a solution containing a precipitant, or by gradually altering the ionic strength orpH. Given potential limitations on the available protein quantity, this approach often utilizes small volumes ($< 50\ mm^3$), for which various microdialysis techniques are available.

3.3.3.2 Vapour Diffusion

Vapor diffusion relies on inducing supersaturation in the sample through controlled equilibration in the vapor phase. In the hanging drop method, a microdroplet (2–20 mm) of protein is placed on a glass coverslip. The coverslip is then inverted

and positioned over a sealed reservoir containing a precipitant solution, with the droplet initially having a lower precipitant concentration than that in the reservoir. Vapor diffusion gradually increases the concentration of the protein solution. Due to the small volumes involved, this method is well-suited for screening a large number of different conditions.

Once crystals are produced, they may be insufficiently sized for examination. In such cases, larger crystals can be generated by utilizing a minute crystal to initiate supersaturation in a protein solution, prompting the growth of a larger crystal. Upon preparation, the delicate crystal is placed inside a quartz or glass capillary tube. A drop of either the mother liquor (the solution from the crystallization process) or a stabilizing solution is drawn into one end of the capillary tube to hinder the dehydration of the crystal. The tube is then sealed, and the crystal is exposed to an X-ray beam. As the X-ray wavelength matches the planar separation of atoms in the crystal lattice, the crystal acts as a three-dimensional grating, leading to X-ray diffraction. The diffracted X-rays interfere in both phase and out of phase, generating a diffraction pattern.

The technology for capturing diffraction patterns has progressed significantly. Initially, traditional diffractometers and photographic film were utilized, followed by weeks of data collection involving the wet development of the film and digital scanning. In contrast, modern area detectors can gather data in less than 24 h. Despite obtaining the diffraction pattern, determining the crystal structure necessitates knowledge of both amplitude and phase for each diffraction maximum. As phases are not directly measurable, the isomorphous replacement (MIR) method is frequently employed. This method involves crystallizing the protein in the presence of various heavy metal ions (e.g., Hg, Cu, Mn), allowing for phase estimation by comparing diffraction patterns.

A contemporary approach involves cloning the protein into a methionine auxotroph and cultivating this strain with selenomethionine, an analogue of methionine containing selenium. Selenomethionine, integrated into the protein, serves as the heavy metal derivative, simplifying the process of obtaining heavy metal derivatives for phase estimation. Following the collection of diffraction data and phase information, a computer processes this data to construct an electron density map. This map, along with the known protein sequence, is used to fit the protein's structure into the electron density map using computer graphics, resulting in a three-dimensional model.

Historically, concerns arose about whether the crystal's rigid structure might vary from the more adaptable structure in a free solution. Experiments involving the diffusion of substrate into an enzyme crystal have tackled these concerns, showing that there is ample mother liquor within the crystal to keep the substrate in solution, allowing the observation of enzymatic reactions. A recent development in structural biology is the use of Nuclear Magnetic Resonance (NMR) to determine the solution structure of proteins. While this method is currently applicable to proteins up to about 20,000 kDa, ongoing advancements may extend its suitability to larger proteins. Despite eliminating the need for crystallization, NMR methodologies and data analysis are currently as intricate and time-consuming as those for X-ray crystallography.

3.4 Factors Affecting Protein Integrity

Proteins, being inherently fragile molecules, are susceptible to denaturation, which involves the loss of their native structure due to unfolding. Several factors influence protein stability, and one crucial aspect is the effect of temperature on protein structure and stability.

3.4.1 Effects of Temperature on Protein Structure and Stability

Elevated temperatures result in increased kinetic energy, causing molecules, including proteins, to vibrate rapidly and with greater force. This heightened molecular motion disrupts the hydrogen bonds and other nonpolar hydrophobic interactions crucial for maintaining the native structure of proteins, ultimately leading to denaturation. Optimal temperature conditions are essential for preserving protein stability and minimizing temperature-induced denaturation.

The study by Andlinger et al. (2021b) explored the influence of protein interactions on the aggregation mechanism of patatin-rich potato protein isolate (PPI) under different heating conditions. The research revealed that disulfide-linked aggregates could form when initial aggregation occurred through non-covalent bonds.

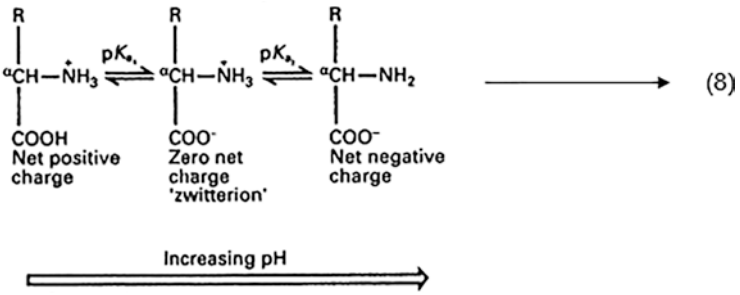
Moreover, the techno-functional properties of PPI aggregates can be altered by varying heating conditions and pH levels. For instance, aggregates with high exposed hydrophobicity prove beneficial for stabilizing air/water interfaces (Dombrowski et al. 2017) and oil/water interfaces (Delahaije et al. 2013). However, when used as an oleogelator, more hydrophilic particles are preferred (de Vries et al. 2017). These aggregates also serve as building blocks for hydrogels, and the microstructure of PPI gels can differ significantly depending on the pH conditions (Andlinger et al. 2021a). Fast aggregation and a high amount of exposed hydrophobicity lead to the formation of unordered, particulate gels. Conversely, conditions with high disulfide bond formation and low exposed hydrophobicity result in finely stranded gels.

3.4.2 Impact of pH on Protein Conformation and Function

Möller et al. (2012) conducted Small-Angle X-ray Scattering (SAXS) studies on concentrated lysozyme solutions with high ionic strength, investigating the impact of temperature and pressure on protein conformation. The conditions examined in their research pertained to the native protein without significant conformational changes. Understanding the intermolecular interaction potential of proteins under the influence of factors such as temperature, pressure, protein concentration, and salt concentration is crucial for comprehending processes like protein aggregation, fibrillation, crystallization, and overall protein phase behavior. Notably, proteins possess both amino and carboxyl groups, leading to the ionization of amino acids at all pH values. Consequently, a neutral species indicated by the general formula does

not exist in the solution, regardless of the pH. This characteristic has significant implications for the behavior and function of proteins in various physiological and experimental conditions.

An amino acid is present as a cation, at low pH values, whereas at higher pH values, it existence is anionic, Eq. (3.8). At a specific intermediate pH, the amino acid carries no net charge, even though it remains ionized, and is termed a zwitterion. Both in the crystalline state and in aqueous solution, amino acids are predominantly found in this zwitterionic form. This imparts upon them physical properties characteristic of ionic compounds, including high melting and boiling points, water solubility, and low solubility in organic solvents like ether and chloroform.



3.8

Source: Walker and Wilson 2010

The pH at which the zwitterion is prevalent in a water-based solution is known as the isoionic point. At this pH, the number of negative charges resulting from the ionization of the carboxyl group equals the number of positive charges obtained through proton acceptance by the amino group. For amino acids, this corresponds to the isoelectric point (pI), where the molecule carries no net charge, making it electrophoretically stationary. The numerical value of the isoelectric point for a specific amino acid is related to its acid strength (pKa values) through the following equation, Eq. (3.9):

$$\mathrm{pI} = \frac{\mathrm{pK_{a1}} + \mathrm{pK_{a2}}}{2} \tag{3.9}$$

where $\mathrm{pK_{a1}}$ and $\mathrm{pK_{a2}}$ are equal to the negative logarithm of the acid dissociation constants, $\mathrm{K_{a1}}$ and $\mathrm{K_{a2}}$.

The ionization behavior of amino acids is not solely confined to the amino and carboxyl groups in their main structure. Alternatively, the side chain (R) in the general formula of an amino acid may contain a diverse chemical group capable of ionizing at a specific pH. Examples include a phenolic group (tyrosine), guanidino group (arginine), imidazolyl group (histidine), and sulphydryl group (cysteine). This leads to substantial differences in the ionization state of amino acids (acidic, basic, neutral) at particular pH values. Even within a specific group, minor variations arise due to the precise nature of the R group. These distinctions are exploited in techniques such as electrophoresis and ion-exchange chromatography for separating amino acid mixtures, as commonly found in protein hydrolysates.

Proteins, formed through the condensation of the α-amino group of one amino acid with the carboxyl of an adjacent amino acid, involve the amino and carboxyl groups in peptide bonds. Except the two terminal amino acids, these groups are no longer ionizable within the protein. However, the sulphydryl, carboxyl, guanidino, phenolic, imidazolyl, and amino groups in the side chains remain free to ionize. Proteins fold in a manner that positions most of these ionizable groups on the outer surface, enabling interaction with the surrounding aqueous environment. Some of these groups, even within the protein structure, contribute to electrostatic attractions that assist in stabilizing the three-dimensional structure of the protein molecule. The equilibrium between negative and positive entities within a protein affects its physical characteristics, such as solubility and electrophoretic mobility.

The isoionic point of a protein, which signifies the pH where the protein molecule possesses an equal number of positive and negative groups, is generally not identical to its isoelectric point. The isoelectric point, determined experimentally by electrophoretic mobility in buffered solutions, is crucial in practical studies. It represents the pH at which a protein exhibits minimal solubility, as it maximizes the potential for attraction between oppositely charged groups, leading to aggregation and precipitation.

The pH of a solution can influence protein folding or unfolding. Experimental studies on the pH-dependent stability of proteins have been conducted, but few theoretical approaches have addressed the thermodynamic aspects of pH-dependent folding. Molecular dynamics simulations can model pH-dependent effects, predicting and interpreting pKa values of titratable groups within native and unfolded ensembles. The Molecular Transfer Model (MTM), used in conjunction with coarse-grained representations of proteins, offers a way to overcome the limitations of thermodynamic models. The MTM, initially introduced for osmolyte effects on proteins, enables the rapid prediction of thermodynamic properties under various external conditions by processing simulations performed at one solution condition. This allows for a more comprehensive understanding of pH effects on proteins.

3.4.3 Influence of Other Factors on Protein Integrity

3.4.3.1 Pressure

The application of pressure has been demonstrated to nonlinearly affect the intermolecular interactions of proteins in solution, potentially connected to changes in the stability of the second hydration shell of water against hydrostatic pressure. This phenomenon is related to pressure-induced alterations in the water structure in the multi-kbar range. The addition of specific cosolvents, such as trimethylamine-n-oxide (TMAO), known to modify local water structure, can counteract pressure-induced effects. In the pressure range discussed, proteins typically remain in their natively folded state, although oligomeric proteins may tend to dissociate in the low kbar-range.

3.4.3.2 Protease Activity

Proteolytic enzymes, or proteases, released into the buffer environment during cellular disruption, can cleave proteins, leading to a loss of stability and denaturation. To mitigate protease activity, rapid purification procedures, maintaining protein solutions on ice, or incorporating protease inhibitors into the buffer can be effective strategies.

3.4.3.3 Freeze-Thaw

Freeze-thaw cycles, a common storage and usage procedure, can disrupt the natural conformation of proteins, interfere with the precipitation of buffer components, and induce pH variations, all contributing to stability loss and denaturation. Adding glycerol to the buffer and storing in aliquots can help minimize these disturbances.

3.4.3.4 Presence of Heavy Metals

Heavy metal salts, being ionic, interfere with salt bridges in proteins and react with proteins, leading to the formation of insoluble metal-protein salts. Including EDTA buffers helps reduce denaturation by chelating heavy metals, preventing their interaction with proteins.

3.4.3.5 Oxidation

Proteins become unstable when oxidized. Incorporating strong reducing agents, such as DTT, into buffers can prevent protein denaturation caused by oxidation.

3.4.3.6 Dilution Effects

Diluting protein solutions can induce denaturation. Preventing dilution-induced denaturation involves maintaining protein concentrations at levels above 1 mg/mL.

3.4.3.7 Agitation

Vigorous shaking, vortexing, or stirring of protein solutions can disturb stability. Careful handling and avoiding excessive agitation are essential to maintaining protein stability and preventing denaturation.

References

Alvarez C, Lelu P, Lynch SA, Tiwari BK (2018) Optimised protein recovery from mackerel whole fish by using sequential acid/alkaline isoelectric Solubilization precipitation (ISP) extraction assisted by ultrasound. LWT Food Sci Technol 88:210–216. https://doi.org/10.1016/j.lwt.2017.09.045

Andlinger DJ, Bornkeßel AC, Jung I, Schröter B, Smirnova I, Kulozik U (2021a) Microstructures of potato protein hydrogels and aerogels produced by thermal crosslinking and supercritical drying. Food Hydrocoll 112:106305

Andlinger DJ, Röscheisen P, Hengst C, Kulozik U (2021b) Influence of pH, temperature and protease inhibitors on kinetics and mechanism of thermally induced aggregation of potato proteins. Food Secur 10(4):796. https://doi.org/10.3390/foods10040796

Arias-Moscoso JL, Maldonado-Arce A, Rouzaud-Sandez O, Marquez-Rios E, Torres-Arreola W, Santacruz-Ortega H et al (2015) Physicochemical characterization of protein hydroly-

sates produced by autolysis of jumbo squid (Dosidicus Gigas) byproducts. Food Biophysics 10(2):145–154. https://doi.org/10.1007/s11483-014-9374-z

Banerjee S (2018) Glyoxal-induced modification enhances stability of hemoglobin and lowers iron-mediated oxidation reactions of the Heme protein: an in vitro study. Int J Biol Macromol 107:494–501. https://doi.org/10.1016/j.ijbiomac.2017.08.180

Bao Y, Boeren S, Ertbjerg P (2018) Myofibrillar protein oxidation affects filament charges, aggregation and water-holding. Meat Sci 135:102–108. https://doi.org/10.1016/j.meatsci.2017.09.011

Ben-Tal A, Nemirovski A (2002) Robust optimization–methodology and applications. Math Program 92(3):453–480. https://doi.org/10.1007/s101070100286

Bhaskar N, Benila T, Radha C, Lalitha RG (2008) Optimization of enzymatic hydrolysis of visceral waste proteins of Catla (Catla Catla) for preparing protein hydrolysate using a commercial protease. Bioresour Technol 99(2):335–343. https://doi.org/10.1016/j.biortech.2006.12.015

Cao W, Zhang C, Hong P, Ji H, Hao J, Zhang J (2009) Autolysis of shrimp head by gradual temperature and nutritional quality of the resulting hydrolysate. LWT Food Sci Technol 42(1):244–249. https://doi.org/10.1016/j.lwt.2008.05.026

Chi C-F, Wang B, Hu F-Y, Wang Y-M, Zhang B, Deng S-G, Wu C-W (2015) Purification and identification of three novel antioxidant peptides from protein hydrolysate of Bluefin leatherjacket (Navodon septentrionalis) skin. Food Res Int 73:124–129. https://doi.org/10.1016/j.foodres.2014.08.038

da Silva CP, Bezerra RS, Dos Santos ACO, Messias JB, de Castro CROB, Junior LBC (2017) Biological value of shrimp protein hydrolysate by-product produced by autolysis. LWT Food Sci Technol 80:456–461. https://doi.org/10.1016/j.lwt.2017.03.008

de Vries A, Gomez YL, van der Linden E, Scholten E (2017) The effect of oil type on network formation by protein aggregates into oleogels. RSC Adv 7:11803–11812

Delahaije RJBM, Wierenga PA, van Nieuwenhuijzen NH, Giuseppin MLF, Gruppen H (2013) Protein concentration and protein-exposed hydrophobicity as dominant parameters determining the flocculation of protein-stabilized oil-in-water emulsions. Langmuir 29:11567–11574

Dombrowski J, Gschwendtner M, Kulozik U (2017) Evaluation of structural characteristics determining surface and foaming properties of β-lactoglobulin aggregates. Colloids Surf A Physicochem Eng Asp 516:286–295

Elavarasan K, Naveen Kumar V, Shamasundar B (2014) Antioxidant and functional properties of fish protein hydrolysates from fresh water carp (Catla Catla) as influenced by the nature of enzyme. J Food Process Preserv 38(3):1207–1214. https://doi.org/10.1111/jfpp.12081

French KM, Somasuntharam I, Davis M, Somasuntharam E (2016) Self-assembling peptide-based delivery of therapeutics for myocardial infarction. Adv Drug Deliv Rev 96:40–53. https://doi.org/10.1016/j.addr.2015.04.023

Halim N, Yusof H, Sarbon N (2016) Functional and bioactive properties of fish protein hydrolysates and peptides: a comprehensive review. Trends Food Sci Technol 51:24–33. https://doi.org/10.1016/j.tifs.2016.02.007

Hua K, Cobcroft JM, Cole A, Condon K, Jerry DR, Mangott A, Praeger C, Vucko MJ, Zeng C, Zenger K (2019) The future of aquatic protein: implications for protein sources in aquaculture diets. One Earth 1(3):316–329. https://doi.org/10.1016/j.oneear.2019.10.018

Hultin HO, and Kelleher SD (2001) Process for isolating a protein composition from a muscle source and protein composition. Google Patents

Hultin H, Kristinsson H, Lanier T, Park J (2005) Process for recovery of functional proteins by pH shifts. Surimi Surimi Seafood 2:107–139

Intarasirisawat R, Benjakul S, Visessanguan W, Wu J (2014) Effects of skipjack roe protein hydrolysate on properties and oxidative stability of fish emulsion sausage. LWT Food Sci Technol 58(1):280–286. https://doi.org/10.1016/j.lwt.2014.02.036

Jamil N, Halim N, Sarbon N (2016) Optimization of enzymatic hydrolysis condition and functional properties of eel (Monopterus Sp.) protein using response surface methodology (RSM). Int Food Res J 23(1):1–9

Khan S, Rehman A, Shah H, Aadil RM, Ali A, Shehzad Q, Ashraf W, Yang F, Karim A, Khaliq A, Xia W (2020) Fish protein and its derivatives: the novel applications, bioactivities, and their functional significance in foods. Food Rev Intl 38:1607. https://doi.org/10.1080/87559129.2020.1828452

Kobayashi Y, Park JW (2017) Biochemical and physical characterizations of fish protein isolate and surimi prepared from fresh and frozen whole fish. LWT Food Sci Technol 77:200–207. https://doi.org/10.1016/j.lwt.2016.11.027

Laxe FG, Bermudez FM, Palmero FM, Novo-Corti I (2018) Governance of the fishery industry: a new global context. Ocean Coastal Manag 153:33–45. https://doi.org/10.1016/j.ocecoaman.2017.12.009

Lulijwa R, Alfaro AC, Merien F, Meyer J, Young T (2019) Advances in salmonid fish immunology: a review of methods and techniques for lymphoid tissue and peripheral blood leucocyte isolation and application. Fish Shellfish Immunol 95:44–80. https://doi.org/10.1016/j.fsi.2019.10.006

Lynch AJ, Cooke SJ, Deines AM, Bower SD, Bunnell DB, Cowx IG, Nguyen VM, Nohner J, Phouthavong K, Riley B (2016) The social, economic, and environmental importance of inland fish and fisheries. Environ Rev 24(2):115–121. https://doi.org/10.1139/er-2015-0064

Mallikage M (2001) The effect of different cooling system on quality of pelagic species. Department of Fisheries and Aquatic Resources, Colombo

Mohanty B, Mahanty A, Ganguly S, Sankar T, Chakraborty K, Rangasamy A, Asha KK (2014) Amino acid compositions of 27 food fishes and their importance in clinical nutrition. J Amino Acids 269797:1

Mohanty BP, Ganguly S, Mahanty A, Sankar T, Anandan R, Chakraborty K, Venkateshwarlu G (2016) DHA and EPA content and fatty acid profile of 39 food fishes from India. Biomed Res Int 4027437:1

Mohanty BP, Mahanty A, Ganguly S, Mitra T, Karunakaran D, Anandan R (2019) Nutritional composition of food fishes and their importance in providing food and nutritional security. Food Chem 293:561–570. https://doi.org/10.1016/j.foodchem.2017.11.039

Möller J, Schroer MA, Erlkamp M, Grobelny S, Paulus M, Tiemeyer S et al (2012) The effect of ionic strength, temperature, and pressure on the interaction potential of dense protein solutions: from nonlinear pressure response to protein crystallization. Biophys J 102(11):2641–2648. https://doi.org/10.1016/j.bpj.2012.04.043

Morales JL, Nocedal J (2002) Enriched methods for large-scale unconstrained optimization. Comput Optim Appl 21(2):143–154. https://doi.org/10.1023/A:1013756631822

Muhammed MA, Manjunatha N, Murthy KV, Bhaskar N (2015) Design and testing of small scale fish meat bone separator useful for fish processing. J Food Sci Technol 52(6):3520–3528

Nasri R, Younes I, Jridi M, Trigui M, Bougatef A, Nedjar-Arroume N et al (2013) ACE inhibitory and Antioxidative activities of goby (Zosterissessor Ophiocephalus) fish protein hydrolysates: effect on meat lipid oxidation. Food Res Int 54(1):552–561. https://doi.org/10.1016/j.foodres.2013.07.001

Nyboer EA, Liang C, Chapman LJ (2019) Assessing the vulnerability of Africa's freshwater fishes to climate change: a continent-wide trait-based analysis. Biol Conserv 236:505–520. https://doi.org/10.1016/j.biocon.2019.05.003

Petrova I, Tolstorebrov I, Eikevik TM (2018) Production of fish protein hydrolysates step by step: technological aspects, equipment used, major energy costs and methods of their minimizing. Int Aquat Res 10(3):223–241. https://doi.org/10.1007/s40071-018-0207-4

Pojić M, Mišan A, Tiwari B (2018) Eco-innovative Technologies for Extraction of proteins for human consumption from renewable protein sources of plant origin. Trends Food Sci Technol 75:93–104. https://doi.org/10.1016/j.tifs.2018.03.010

Ramakrishnan V, Ghaly A, Brooks M, Budge S (2013) Extraction of proteins from mackerel fish processing waste using Alcalase enzyme. Bioprocess Biosyst Eng 3(2):1–9

Rehman A, Tong Q, Jafari SM, Assadpour E, Shehzad Q, Aadil RM, Iqbal MW, Rashed MMA, Mushtaq BS, Ashraf W (2019) Carotenoid-loaded Nanocarriers: a comprehensive review. Adv Colloid Interf Sci 275:102048

Sen D (2005) Advances in fish processing technology. Allied Publishers, Mumbai
Senphan T, Benjakul S, Kishimura H (2014) Characteristics and Antioxidative activity of Carotenoprotein from shells of Pacific white shrimp extracted using Hepatopancreas proteases. Food Biosci 5:54–63. https://doi.org/10.1016/j.fbio.2013.11.004
Shaviklo AR (2015) Development of fish protein powder as an ingredient for food applications: a review. J Food Sci Technol 52(2):648–661. https://doi.org/10.1007/s13197-013-1042-7
Shaviklo AR, Etemadian Y (2019) Overcoming current challenges in commercial applications of fish protein isolates in food and feed systems: a review. J Food Sci Technol 56:1–10
Srichanun M, Tantikitti C, Kortner TM, Krogdahl A, Chotikachinda R (2014) Effects of different protein hydrolysate products and levels on growth, survival rate and digestive capacity in Asian seabass (Lates Calcarifer Bloch) larvae. Aquaculture 428:195–202. https://doi.org/10.1016/j.aquaculture.2014.03.004
Venugopal V (2005) Seafood processing: adding value through quick freezing. In: Retortable packaging and cook-chilling. CRC, Boca Raton
Walker J, Wilson K (2010) Protein structure purification characterisation and function analysis. In: Principles and techniques of biochemistry and molecular biology. Cambridge University Press, Cambridge, pp 349–404

Derivatives of Structural Proteins

4

Dhanya Pulikkottil Rajan

Abstract

This chapter explores the realms of marine proteins, focusing particularly on marine structural proteins and their derivatives. A thorough examination of different extraction methods for obtaining collagen, gelatin, and enzymes is provided, offering detailed insights into the production processes. Additionally, the discussion extends to protein derivatives sourced from secondary raw materials, such as fish protein hydrolysates and collagen peptide/gelatin hydrolysates. Techniques for production, isolation, and characterization from fish skin, bones, and scales are meticulously explored. Furthermore, alternative methods of collagen extraction, including acid extraction procedure, deep eutectic solvent (DES) extraction, Supercritical fluid extraction (SFE), extrusion, and ultrasound-assisted extraction, are elaborated upon, highlighting their efficacy and applicability. The chapter also comprehensively covers collagen characterization methods, focusing on aspects such as the determination of chemical composition, purity, and secondary structure. Moreover, the discourse extends to gelatin extraction methods and the diverse functions of protein derivatives, providing a holistic understanding of their utilization and potential applications within the marine protein industry.

Keywords

Collagen · Extraction methods · Marine proteins · Gelatin · Fish protein hydrolysates

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4.1 Introduction

Proteins are made by linking 20 natural amino acids through amide bonds. Beyond these 20, there exist amino acids, like L-3,4-dihydroxyphenylalanine (DOPA), hydroxyproline (Hyp), dityrosine, and selenomethionine, which are not directly synthesized from ribosomes. Instead, they undergo posttranslational modifications. These nonribosomal peptides and amino acids often play crucial roles in the structure and function of proteins. Frequently, structural proteins exhibit a distinctive amino acid sequence that repeats, facilitating the formation of a higher-order structure through intermolecular and/or intramolecular hydrogen bonding (Askarieh et al. 2010).

An illustration of this is the disulfide bond, created between two cysteine residues, which has the capacity to induce dimerization and hierarchical structures within proteins (Hang et al. 2010). However, in a general context, the amino acid sequence and the resulting structure are contingent on the type of structural protein, making it challenging to identify such proteins solely based on sequence or structure. This challenge becomes even more intricate when considering non-natural sequences generated through gene recombination techniques and chemical synthesis (Tsuchiya and Numata 2017). Defining a structural protein becomes particularly challenging under these circumstances.

A structural protein can be characterized as "a protein possessing a characteristic amino acid sequence or motif that repeats and forms a skeleton or contributes to the mechanical properties of a living organism, cell, or material." These structural proteins may take on either globular or fibrillar forms. In most instances, these motifs are anticipated to give rise to higher-order structures through intermolecular or intramolecular interactions, thereby influencing the expression of physical characteristics (Numata 2020).

4.2 Marine Proteins

Fish and shellfish provide substantial nutritional value, and products derived from fisheries are known for their delectable taste. The awareness of the health advantages associated with fish consumption is on the rise, contributing to a continually growing demand for fish. However, fish, in contrast to meat from land animals, is highly susceptible to spoilage due to factors such as a nearly neutral postmortem pH, limited glycogen reservoir, reduced connective tissue content, and elevated moisture content. The categorization of proteins in fish muscle, outlined by Huss (1995), divides them into three distinct groups. They are given below.

1. *Sarcoplasmic proteins* (enzymes, myoalbumin, and globulin) are soluble in neutral salt solutions of low ionic strength (<0.15M). This fraction represents 25–30% of the total proteins. Approximately 100 different proteins have been identified in the sarcoplasmic fraction.
2. *Structural proteins*—myosin, actin, tropomyosin, and actomyosin, which collectively make up 60–70% of the total protein content, are structural proteins.

They exhibit solubility in neutral salt solutions of relatively high ionic strength (0.5M and above).
3. *Stroma proteins*—accounting for around 3% in teleostei and approximately 10% in elasmobranchii (in contrast to 17% in mammals), encompass connective tissue, collagen, and elastin.

4.3 Marine Structural Proteins and Its Derivatives

4.3.1 Collagen Extracted from Fish Tissue

Collagen, a structural protein predominantly found in the skin and bones of all animals, stands as the most abundant protein sourced from animals, constituting around 30% of the total animal protein. Comprising three α-chains intertwined to create a triple-helix structure, collagen resides in the connective tissue matrix that forms the framework of bones, joints, cornea, blood ducts, placenta, and skin, which are examples of tissues where collagen is abundantly present. While various collagen types exist, type I collagen makes up 90% of the body's collagen protein, rich in amino acids such as hydroxyproline, proline, alanine, valine, and glycine that are found in proteins (Burghagen 1999). Glycine comprises one-third of collagen's total amino acid content, followed by hydroxyproline and proline, making up another third. Due to collagen's unique structural properties, there is a growing interest in consuming collagen directly through derivatives that can be digested with ease. Globally, this interest has been embraced by the nutraceutical industry, particularly in developing nations. Collagen is currently utilized in various cosmetic and pharmaceutical products, due to its structural function and compatibility with the human body. Main role of collagen in the cosmetics industry is to produce skin lotion, forming a protective film of higher quality to hydrate and soothe the skin. Collagen's immense potential has significant implications for anti-aging treatments. Beyond that, collagen finds diverse applications in cosmetic and burn surgery, particularly as dermal fillers for bone and skin reconstruction. Collagen gels hold clinical importance in preparing "artificial skin" for treating major wounds. Injectable collagen hydrogels have been successfully employed for drug delivery, soft-tissue augmentation, and hard-tissue augmentation. Microfibrous collagen sheets serve as effective drug carriers for cancer treatment. Moreover, collagen is an essential component in various dental treatments and orthopedic, recently emerging as a supplement for joint mobility.

4.3.2 Gelatin Extracted from Fish Tissue

Gelatin is a soluble polypeptide. It is acquired by unfolding the insoluble collagen. The process involves breaking cross-linkages among collagen's polypeptide chains, coupled with a partial cleavage of intra-polypeptide bond chains. Collagen-containing tissues undergo mild degradative treatments, involving acid or alkali

exposure followed by heating with water. This systematically breaks down the fibrous structure of collagen irreversibly, resulting in the production of gelatin. Remarkably, gelatin is the only protein-based food material capable of reversible gelation and melting at a temperature lower than the human body temperature. With exclusive functional properties and easy to obtain at an affordable cost, gelatin is widely used as a pharmaceutical and food ingredient. Bones and fish skins offer an alternative source for gelatin production, addressing waste disposal challenges and adding value. However, it's worth noting that gels derived from fish gelatins may exhibit lower stability and inferior rheological properties compared to those from mammalian gelatins. Despite this drawback, fish gelatin possesses distinctive qualities, such as enhancing the smell and taste of a product, along with fewer inherent off-flavors and odors than commercial pork gelatin. These characteristics open up new possibilities for product developers.

4.3.3 Fish Enzymes

Fish visceral waste represents a valuable source of enzymes with broad potential applications across various sectors, ranging from laundry to pharmaceuticals (Simpson and Haard 1987). The enzymes present in fish viscera differ from those in the digestive systems of terrestrial animals, making them suitable for distinct applications. Fish pepsins, for instance, exhibit activity at lower temperatures and have a higher pH optimum compared to pepsins from land animals. Additionally, fish pepsins resist autolysis at low pH (Raa 1990), a characteristic that sets them apart. According to Gildberg and Overbø (1990), the variations in properties between fish pepsins and those from other sources are attributed to differences in amino acid sequence and composition. Fish enzymes find utility as processing aids in various applications, including:

- Production of protein hydrolysates
- Caviar production from different fish species
- Squid skin removal
- Scallops cleaning
- Fish descaling
- Milk coagulation
- Cheese production

4.3.4 Hemoproteins

Hemoproteins are intricate proteins consisting of a protein molecule and a non-protein compound known as a prosthetic group. Hemoglobin and myoglobin fall into the hemoprotein category, playing roles in transporting oxygen in the blood and tissues of animals, respectively. The blood and muscle tissue discards can be used for extracting heme containing portion in a hemoprotein. The retrieved material may serve as an iron supplement or act as a chemical substrate for producing the

pigment in cooked and cured meat. In the process of producing meat hydrolysates, hemin can be obtained as a by-product.

4.3.5 Carotenoproteins

Carotenoproteins as well as carotenoids represent additional classes of compounds present in the outer skeleton of shellfish like prawns and the skin/flesh of fishes. These compounds are not manufactured within the body but are obtained through the food chain (Haard 1992). Similar to hemoproteins, carotenoids consist of a protein component and a non-protein prosthetic group. The isolation of carotenoproteins and carotenoids from discarded materials in shellfish processing has been documented. The significance of these compounds in industrial applications is underscored by their incorporation into the feed formulations of certain aquacultured and ornamental fishes (Shahidi et al. 1993).

4.4 Protein Derivatives from Secondary Raw Material

4.4.1 Fish Protein Hydrolysates (Bioactive Peptides)

Fish muscle proteins, besides being highly nutritious, can be utilized to create fish protein hydrolysates comprising bioactive peptides with treasured pharmaceutical and nutraceutical potential. Fish protein hydrolysates (FPHs) contain amino acids and peptides obtained by treating proteins from fish meat or processing waste with enzyme proteases. These peptides with greater than 6000 Da molecular weight contain amino acid residues ranging from 2 to 20. They exhibit high bioactivity and can serve as functional food ingredients/nutraceuticals contributing to human health and disease prevention. Major pharmaceutical companies are increasingly investing in bioactive peptide research to explore therapeutic possibilities.

The proximate composition of fish protein hydrolysate varies with raw materials (head, bone, skin, viscera), processing type, drying method, hydrolysis extent, and any other pretreatment of raw materials. The chemical composition of fish protein hydrolysates plays a crucial role in supplying essential nutrients for maintaining health. The amino acid composition of protein hydrolysates, produced from different raw materials using various enzyme sources under different hydrolysis conditions, is expected to vary. Fish protein hydrolysates (FPHs) are rich in essential amino acids, characterized by elevated levels of glutamic and aspartic acid. Additionally, non-essential amino acids are present, and the inclusion of aromatic amino acids in fish frame protein hydrolysates has been documented, highlighting their suitability as a source of essential amino acids (Chalamaiah et al. 2012).

Some fish protein hydrolysate products/peptides are marketed as health supplements in developed countries, demonstrating specific health benefits beyond nutritional value. These hydrolysates or peptides have been proven to possess anti-obesity,

antihypertensive, antioxidant, anticoagulation, immune modulation, antimicrobial, and anticancer properties (Elavarasan et al. 2014, 2016).

Fish protein hydrolysates exhibit solubility over a wide pH range, making them suitable for a variety of products. They also enhance emulsifying, foaming, water-holding, and oil-binding properties. However, the degree of hydrolysis is a key factor influencing functional properties, with extensive hydrolysis potentially leading to a loss of functionality. Achieving a critical degree of hydrolysis is essential to preparing protein hydrolysates for specific functional roles as ingredients (Elavarasan et al. 2016; Gajanan et al. 2016).

4.4.2 Collagen Peptide/Gelatin Hydrolysate

Collagen peptide, also known as "collagen/gelatin hydrolysate" or "hydrolyzed collagen," is derived from collagen and gelatin, which are high-molecular-weight proteins of around 300 kDa. Due to their large size, these proteins are challenging for digestion and thus are less available to mankind for doing biological functions. In current years, significant consideration has been devoted to creating small molecular weight peptides from native collagen to enhance their biological activities. This is achieved through the hydrolysis process, wherein natural collagen/gelatin molecules are cleaved into smaller fragments. The hydrolysis process transforms collagen from near 300 kDa to small peptides with an average molecular weight of ˂5 kDa. The result is the whole dissolution of the peptide mixture in cold water, expanding the application prospects of collagen peptide. Small peptides are required for pharmaceutical and nutraceutical applications, while large peptides are preferred for the modification of function of food products. Standardizing collagen manufacturing processes is an essential undertaking within the nutraceutical and health food sector.

From a nutritional standpoint, peptides are more bioavailable than proteins or free amino acids and are less allergenic than their native proteins (Otani et al. 1990). Collagen peptides, besides offering nutritional benefits, have been shown to enhance the absorption of vitamins and minerals. Consequently, combined formulations of collagen peptides with minerals and vitamins are emerging in the market. Bioactive collagen peptides exhibit a wide range of physiological functions, including antihypertensive, antioxidative, anticancer, immunomodulatory, antimicrobial, mineral binding, antithrombotic, and hypocholesterolemic effects (Gòmez-Guillèn et al. 2002).

4.5 Production, Isolation, and Characterization Techniques

4.5.1 Marine Collagen Extraction

In vertebrates, including mammals, fish, and birds, there are 28 different types of collagen coded by at least 45 different genes (Meyer 2019). Collagen, the primary

structural protein in the extracellular matrix (ECM), constitutes approximately 25% to 35% of the body's total protein content. Among the 28 categories of collagen, 80–90% of the collagen in the body comprises types I, II, and III. Type I, the most abundant, is found in bones, skin, tendons, and organs. Type II is present in cartilage, and type III is found in reticular fibers, blood, and skin. Collagen type III is also located in the vessel walls, skin, and reticular fibers of the lungs, liver, and spleen. Types IX, XIV, and XIX (FACIT: fibril-associated collagens with interrupted triple helices) are found in low amounts associated with the fibril-forming types, while types IV, XIX, and XVIII are located in basement membranes of cell membranes. Types I to IV are the most prevalent in vertebrates.

A solitary collagen molecule measures roughly 300 nm in length and has a diameter of 1.5–2 nm. Multiple collagen molecules aggregate to form larger collagen structures, such as fibrils (Paul and Bailey 2003). Collagen has a triple-helical α-domain(s) structure known as "tropocollagen," consisting of three separate chains. These chains, forming a right-handed triple helix or "superhelix," are coiled around each other in a rope-like manner, stabilized by numerous hydrogen bonds (Shoulders and Raines 2009; Paul and Bailey 2003). The coiling process necessitates the presence of a glycine (Gly) residue in every third amino acid, with proline and Hyp frequently occupying other positions in the chain.

The arrangement follows a repeating design of X-Y-Gly, where X and Y can be any amino acid residues. Proline and Hyp often occupy the X and Y positions, respectively. The Pro–Hyp–Gly tripeptide is the most common in collagen. The presence of Hyp in the X position contributes to the stability of the triple helix, as its hydroxyl group forms essential bonds with the pyrrolidine ring, increasing denaturation temperature and enthalpy. The triple helices of collagen benefit from a network of water molecules, contributing to enhanced thermal stability. The denaturation temperature, which signifies the stability of the collagen triple helix, is influenced by both entropic and enthalpic factors. Interchain hydrogen bonding plays a crucial role, as the NH group of a Gly residue forms a peptide bond with carboxyl groups in an adjacent polypeptide, reinforcing the three chains and providing moderate resistance against attacks by other molecules (Paul and Bailey 2003; Meyer 2019; Yin et al. 2018).

Marine collagens, sourced from fish skin, bone, cartilage, and scales, encompassing both marine vertebrates and invertebrates, exhibit greater bioavailability compared to bovine or porcine collagen. They demonstrate a higher absorption capability, being up to 1.5 times more efficient in entering the body (Khan et al. 2009). This enhanced efficiency is attributed to their low molecular weight and small particle size, resulting in a more rapid bloodstream circulation (Alemán and Martínez-Alvarez 2013). Additionally, marine-based collagens share similarities with conventional bovine and porcine collagen in terms of amino acid composition and biocompatibility (Carvalho et al. 2018).

Fish collagen, specifically sourced from several fish by-products such as skins, fishbone, and scales (Fig. 4.1), offers an environmentally friendly solution as it utilizes materials that are daily consumed in different countries in the world. Moreover, these by-products contribute to a substantial amount of waste, accounting for

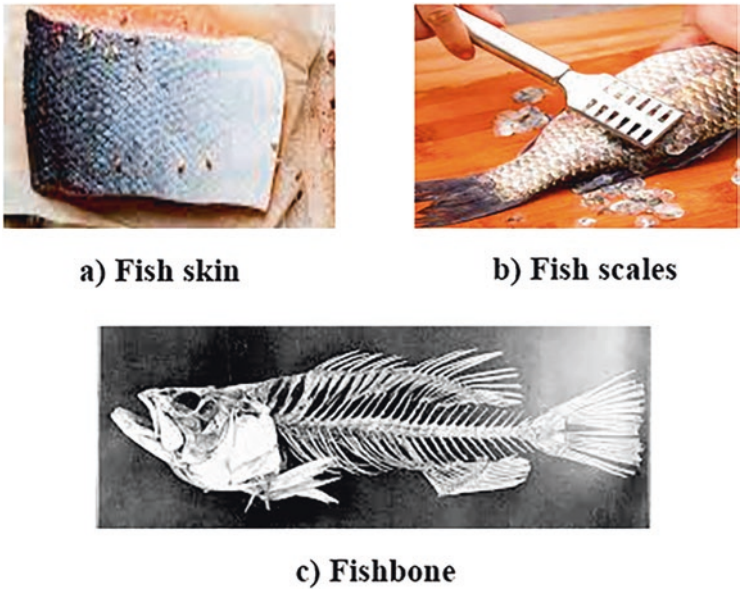

Fig. 4.1 Sources of collagen. (**a**) Fish skin. (**b**) Fish scales. (**c**) Fishbone

50–70% of the original raw materials generated from fish shops and processing factories (Blanco et al. 2017).

4.5.1.1 Extraction from Fish Skin

Fish skin typically contains type I collagen with a high degree of purity, approximately 70%, depending on the species' age and season (Chinh et al. 2019). Collagen derived from fish skin exhibits excellent water retention capacity, retaining about 6% of its weight when exposed to 63% humidity for 24 h. Moreover, it shows no irritant potential, making it suitable for dermal applications (Cumming et al. 2019).

Studies on collagen from different fish species reveal variations in denaturation temperatures. Blanco et al. (2017) found collagen from the skin of two teleost species and two Chondrichthyes species to have denaturation temperatures between 23 and 33 °C, while collagen from codfish skin denatured around 16 °C, possibly influenced by the species' habitat (Sionkowska and Kozlowska 2014). However, collagen from the skin of mackerel, tilapia, catfish, and pomfret requires a low extraction temperature (slightly below 13.26 °C) and a long extraction time (74 h) and yields around 2.27% based on dry mass content (Liu and Huang 2016).

Wijaya et al. (2020) explored the allergenic properties of collagen derived from Parang-Parang fish skin, isolated using 0.1M NaOH in 12 h and hydrolyzed with 0.5M acetic acid (AcOH) before the experiment. The protein content excluding collagen was 0.2163 mg/mL, yielding 1.915%. Govindharaj et al. (2019) explored the application of eel skin-derived collagen (type I) for 3D printing applications,

Fig. 4.2 Collagen extraction methods (Jafari et al. 2020)

achieving a final collagen yield of approximately 4.2%. Similar results were described in which the yield of collagen processed from eel fish was 4.7% (Veeruraj et al. 2013).

Ahmed et al. (2018) explored the use of bacterial collagenolytic proteases (CP) as an alternative method for extracting collagen from fish skin. Utilized two bacteria strains, *Bacillus cereus* FORC005 and *Bacillus cereus* FRCY9-2, for the production of CP. The total yield of collagen through bacterial treatments, combined with acid-soluble collagen, was 188 and 177 g/kg, respectively. These yields surpassed those obtained from acid extraction only (134.5 g/kg).

Another method for collagen extraction from fish skin involves the use of water acidified with CO_2, applied in the isolation of collagen from Atlantic cod (*Gadus morhua*) (Sousa et al. 2020a, b). Collagen extracted with acidified water exhibited a total content of proline-like amino acids at 151 per 1000 residues, with an extraction yield of 13.8% (w/w) (Sousa et al. 2020a, b). Collagen extraction method is depicted in Fig. 4.2.

4.5.1.2 Extraction from Fish Scale

Fish scales, a substantial by-product of the fish processing industry, have garnered attention in recent studies (Van De Water et al. 2013; Jeevithan et al. 2014; Liang et al. 2014) as a potential source of collagen. Collagen obtained from fish scales is suggested to exhibit properties typical of type I collagen, consisting of two-1 chains and one-2 chain. An investigation into collagen from tilapia (*Oreochromis* sp.) scales revealed a high denaturation temperature (57.9–79.0 °C), likely attributed to its higher amino acid amount and increased intra/interchain bonds, including hydrogen bonds, dipole–dipole bonds, ionic bonds, and Van der Waals interactions (Jeevithan et al. 2014). In contrast, collagen obtained from fresh carp (*Cyprinus carpio*) scales displayed a lower denaturation temperature (32 °C) as reported by Liang et al. (2014). Collagen extraction from the scales of tilapia, catfish, pomfret, and mackerel necessitates a higher extraction temperature (ranging from 16.6 to 19.03 °C) and an extended extraction period (77.51 h). This results in lower extraction yields (0.13%) compared to fish skin (4.3%), as observed by Chen et al. (2016a, b).

Despite the challenges in extraction, collagen from fish scales has demonstrated proper water absorption (13.3%) and retention properties (15%), making it suitable for medical and therapeutic applications (Jeevithan et al. 2014). Wound dressings which have a collagen base, including paste and sheet formulations derived from the scales of tilapia and gray mullet, have demonstrated remarkable antimicrobial activity against *E. coli* and *S. aureus*, as assessed through a disk diffusion method. Additionally, these wound dressings have shown a high capacity for wound closure,

reaching up to 99.63%. This suggests that fish scale collagen plays a significant role in accelerating re-epithelialization (Van De Water et al. 2013; Shalaby et al. 2020).

However, it's worth noting that fish scales contain a high amount of calcium, constituting 16–59% of the mineral content by weight. To address this, decalcification is necessary, typically performed using ethylenediaminetetraacetic acid (EDTA) (Gauza-Włodarczyk et al. 2017).

4.5.1.3 Extraction from Fish Bones

Collagen derived from fish bones exhibits properties characteristic of type I collagen, comprising two-1 chains and one-2 chain (Duan et al. 2009; Kimura et al. 1991). An experiment on collagen extracted from Tilapia (*Oreochromis mossambicus*) bones indicates a denaturation temperature of 32.5 °C (Yunoki et al. 2003). Unlike fish scales and skin, collagen from the bones of mackerel, tilapia, catfish, and pomfret requires a higher temperature for extraction (16.6–19.03 °C) but the duration for extraction is shorter (73.16 h), resulting in lower extraction yields (0.64%) compared to fish skin (Chen et al. 2016a, b).

Ramli et al. (2019) highlighted that collagen extracted from *Lutjanus* sp. bone, with a yield of 4.535% and a protein concentration of 8.815 mg/mL, exhibits potential as a natural anticancer agent. The application of high-intensity pulsed electric fields (PEF) is a significant technique for extracting collagen from fish bones, as emphasized by Shavandi et al. (2019a, b). In a study by He et al. (2017), a combined extraction method involving semi-bionic extraction (SBE) and PEF treatments was employed for isolating calcium, chondroitin, and collagen from waste fish bones. The authors achieved an extraction of 3.87 mg/mL of collagen using PEF of 22.79 kV/cm. This combined technique was deemed efficient for isolating collagen, calcium, and chondroitin from fishbone.

In a subsequent study, the same group (He et al. 2019) attained a maximum collagen yield of 16.13 mg/mL from fish bones using 1% pepsin and a PEF strength of 20 kV/cm. Desalting is recommended as an essential process in bone collagen extraction due to its high hydroxyapatite and calcium content, which can be removed by EDTA or HCl during pretreatment. However, using HCl may degrade the collagen (Yunoki et al. 2003).

4.5.1.4 Extraction from Fish Cartilage

Collagen extracted from fish cartilage is primarily composed of type II collagen, with minor quantities of other types such as type IX and type XI found in the nasal cartilage of Hoki (*Macruronus novaezelandiae*) (Cumming et al. 2019). In some species, such as *Sphyrna lewini*, *Dasyatis akjei*, and *Raja porosa*, type I collagen is found in the cartilage (Brazee and Carrington 2006). Type I collagen was identified in acid/salt-solubilized collagen obtained from the cartilage of Amur sturgeon (*Acipenser schrenckii*), while type II collagen, along with other minor types, was found in pepsin-solubilized collagen (Wu et al. 2019a, b).

Hoki cartilage-derived collagens share similar chain assembly, amino acid composition, and structure with mammalian cartilage collagens, making them potential candidates for biomaterials in the treatment of cartilage-related diseases (Lucas

et al. 2002). Jeevithan et al. (2014) evaluated the physicochemical and antioxidant properties of collagen isolated from silvertip shark (*Carcharhinus albimarginatus*) cartilage. Type II acid-soluble collagen (ASC), pepsin-solubilized collagen (PSC), and type II gelatin were extracted from this cartilage. Although the denaturation temperature of type II gelatin was higher than that of PSC and type II ASC, PSC exhibited higher antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl radicals and greater reducing power. The collagens isolated from the silvertip shark are considered suitable candidates for biomedical applications due to their enhanced antioxidant activity.

In a separate study, Luo et al. (2018) obtained ASC and PSC from cartilages of Siberian sturgeon (*Acipenser baerii*) with yields of 27.13 ± 1.15% and 14.69 ± 0.85%, respectively. The results suggest that collagens from this cartilage could serve as suitable alternatives to mammal type II collagens.

Fish cartilage collagen generally exhibits a lower denaturation temperature compared to bovine collagen, ranging from 26.3 °C to 35.9 °C. This temperature variance is attributed to the habitat of the species; for instance, Hoki collagen's lower denaturation temperature aligns with its cold-water habitat (Brazee and Carrington 2006; Qin et al. 1997; Qin and Waite 1995). Collagen extracted from chum salmon (*Oncorhynchus keta*) exhibits denaturation at 19 °C, whereas shark collagen denatures at a higher temperature, around 30 °C (Carvalho et al. 2018). The temperature sensitivity of fish collagen may limit its application in certain human medical contexts. Consequently, there is a need for further investigation to identify sustainable alternatives to fish collagen with very high denaturation temperatures, enhancing suitability for biomedical applications and ensuring improved mechanical and thermal stability. Beyond thermal stability, the composition and structure of collagen should closely resemble mammal collagens for optimal performance in biomedical applications.

4.5.1.5 Other Collagen Extraction Methods

Various extraction methods can be employed depending on the marine sources. Nonetheless, the standard collagen isolation process involves three main steps: preparation, extraction, and recovery. The preparation phase typically includes washing, cleaning, separating animal parts, and size reduction through cutting or mincing the samples to facilitate subsequent pretreatment (Jongjareonrak et al. 2005). Following preparation, a mild chemical pretreatment is undertaken to enhance extraction efficacy and eliminate non-collagenous substances. The specific pretreatment method may vary depending on the raw materials and chosen extraction technique, with options including alkaline or acid treatment. Diluted acid or base is often employed in pretreatment to break down the crosslinked collagen in the connective tissue of animals before the actual extraction process (Schmidt et al. 2016). Certainly, during acidic pretreatment, partial hydrolysis occurs, preserving the integrity of the collagen chains (Prestes 2013). In this form of pretreatment, the raw materials are immersed in an acidic solution. The solution permeates the collagen structure, causing it to swell to two or three times its original volume,

resulting in the cleavage of non-covalent inter- and intramolecular bonds (Draget et al. 2000).

The alkaline pretreatment is mostly performed by using sodium hydroxide (NaOH) and calcium hydroxide ($Ca(OH)_2$) for a period that can take from a few days to several weeks (Prestes 2013). However, using NaOH is more convenient due to the higher swelling ability leading to facilitating the extraction of collagen by increasing the transfer rate of the mass in the tissue matrix (Liu et al. 2014). Moreover, prior to the extraction phase, the demineralization of the raw materials is required to enhance collagen extraction efficiency from the part of the body with a high amount of minerals such as bone, cartilage, and scales. Usually, demineralization can be done by using either EDTA or HCl (Kittiphattanabawon et al. 2010; Żelechowska et al. 2010).

Collagen fibers adopt a triple-helix structure with stable inter- and intramolecular hydrogen bonds, rendering them insoluble in water. Hence, specialized extraction methods are essential to improve the solubilization of collagen proteins and achieve their isolation. Acid-solubilized collagen (ASC), pepsin-solubilized collagen (PSC), deep eutectic solvent (DES), and supercritical fluid (SF) extractions are the main techniques outlined in the literature for collagen isolation from fish by-products (Ibáñez and Castro-Puyana 2016; Bai et al. 2017).

Acid Extraction Procedure

Acids, including HCl and AcOH, hydrolyze the triple-helix structure of collagen, solubilizing its single chains in solution. This process results in the depolymerization of heavyweight proteins into shorter peptides with molecular weights ranging from 0.3 to 8 kDa (Bai et al. 2017). The interaction between acids and collagen molecules breaks the crosslinks within the collagen helix, enhancing the extraction efficiency. Therefore, exploring the extraction efficiency using different acids is of significant interest to maximize the purity and yield of the extracted collagen.

Acetic acid (AcOH) is a commonly used compound for collagen extraction from both animal and marine sources (Blanco et al. 2019). The acid extraction solution typically ranges in concentration between 0.5 and 1M, facilitating the breakage of intra- and intermolecular crosslinks without compromising the structure of collagen chains. While many studies employ 0.1 or 0.5M AcOH for fish skin collagen extraction, Tan and Chang (2018) explored the impact of different acids (AcOH, hydrochloric acid, citric acid, and lactic acid with a liquid-to-solid ratio of 1:50), various pH levels (1.8, 2.1, 2.4, 2.7, and 3.0), and different extraction methods (acid, homogenization-aided, and pepsin-aided) on catfish skin collagen extraction.

In their study, the pepsin and homogenization-aided (PHSC) method demonstrated the maximum protein recovery (64.19% at pH 2.4 with HCl). In terms of the acid extraction method, the highest protein recovery rate was observed with HCl at pH 2.4 (42.36%), followed by extraction with AcOH at pH 2.7 (39.45%). This finding contradicted other studies reporting lower collagen extraction yields with HCl compared to AcOH. The discrepancy might be attributed to variations in acid concentrations used and fluctuations in the pH of the mixture during the extraction process.

To investigate the impact of acetic acid concentration on collagen extraction from sole fish skin while maintaining other variables constant, a series of experiments were conducted by Arumugam et al. (2018) with varying acetic acid concentrations. The results revealed a gradual increase in collagen yield as the concentration of acetic acid increased, reaching its peak at 0.6M acetic acid with a yield of 15.968 mg/g. However, concentrations beyond this point led to a decline in collagen yield.

The study conducted by Yang et al. (2018) investigated the interaction between collagen molecules and acetic acid (AcOH) solutions. The aggregated state of collagen molecules was found to be associated with the concentration of AcOH. As the concentration of AcOH increased from 0.1 to 2.0M, the critical aggregation concentration of collagen also increased, ranging from 0.518 to 1.581 mg/mL. The observed shear-thinning behavior in steady shear tests across all samples indicates a favorable interaction between collagen molecules and the acidic solvent, reinforcing their suitability as 3D printing bioinks.

As the concentration of acetic acid (AcOH) increased, there was a corresponding increase in the flowability of collagen. This enhanced flowability can be attributed to a decrease in viscosity. It is noteworthy that, in addition to AcOH, alternative acids such as citric acid and lactic acid have been employed for collagen extraction, as reported by Tan and Chang (2018).

While acid extraction methods are commonly employed for collagen isolation, they have drawbacks such as high acidity, prolonged processing time, and elevated temperatures, which can lead to significant degradation of soluble collagen chains. Consequently, researchers have been exploring innovative and sustainable extraction techniques to achieve higher collagen yields while using cost-effective and less toxic materials. The goal is to develop processing technologies that preserve target peptides more effectively, addressing the limitations associated with traditional acid extraction methods.

Deep Eutectic Solvent (DES) Extraction

The passage describes the use of Deep Eutectic Solvents (DES) as a method for the extraction of collagen from marine by-products. DES is a mixture of two compounds, one serving as a hydrogen bond acceptor (HBA) and the other as a hydrogen bond donor (HBD). The DES method utilizes natural components such as choline chloride, oxalic acid, urea, and ethylene glycol, making it abundant, low toxic, and biodegradable. The DES method is particularly suitable for extracting valuable chemicals from animal, plant, and marine by-products.

The study by Bai et al. (2017) found that the combination of choline chloride (CC) and oxalic acid (OA) as a DES mixture was the most effective. It showed extraction productivities near 90% for collagen from cod skins. This efficiency was significantly higher compared to AcOH extractions from the skin of tilapia. The ratio of CC to OA in the DES mixture was found to influence the extraction efficiency, with an optimum ratio of 1/1. Higher temperatures in the DES extraction method were observed to increase collagen extraction efficiency ultimately reaching a plateau between 65 and 75 °C. The increased temperature also improved the purity of the extracted collagen. Additionally, the study compared collagen yield in

three isolation and extraction approaches (pepsin-aided AcOH, acid, and DES) from different fish skins, and DES extraction showed the highest performance with a yield of 99.72%.

The extraction time and solid-to-liquid (S/L) ratio were also considered in the comparison of collagen yield among the three approaches. DES extraction demonstrated higher collagen yield compared to acid extraction procedures, and the yield amplified early and then remained persistent or raised with a low slope. The study emphasizes the effectiveness of DES as a sustainable substitute for collagen extraction from marine by-products, contributing to high yields and purity.

Supercritical Fluid Extraction (SFE)

Deep Eutectic Solvent (DES) is composed of two compounds, one serving as a hydrogen bond acceptor (HBA) and the other as a hydrogen bond donor (HBD). Utilizing natural components like choline chloride, ethylene glycol, urea, oxalic acid, and DES is an effective method for extracting valuable chemicals from various by-products. Researchers have found the mixture of oxalic acid and choline chloride to be particularly efficient, showing extraction efficiencies close to 90% for collagen from cod skins. This makes it a sustainable alternative for collagen peptide isolation from marine by-products.

Moreover, the ratio of choline chloride to oxalic acid in DES significantly impacts collagen extraction efficiency. Various ratios were tested, and an increase in oxalic acid initially led to higher extraction efficiency until a ratio of 1/1 was reached, beyond which the yield slightly decreased. Extraction temperature also plays a crucial role, with efficiency increasing steadily with temperature until reaching a plateau. Higher temperatures improve the purity of the obtained collagen, but the efficiency decreases beyond a certain point.

Comparative studies have shown that DES extraction outperforms traditional acid extraction methods in terms of collagen yield. Additionally, supercritical fluid extraction (SFE) has gained popularity as a green extraction technique, offering advantages such as enhanced selectivity, better fractionation capabilities, higher yields, and reduced environmental impact compared to classical extraction processes (Ibáñez and Castro-Puyana 2016).

Extrusion and Ultrasound-Assisted Extraction of Collagen

Extrusion cooking, characterized by high-temperature short-time (HTST) and high shear force processes, has been utilized for years in the production of various food and animal feed products, including forming and expanding cereals, cooking in the food industry, and texturizing proteins. The extrusion process involves multiple reactions such as grinding, hydration, gelatinization, shearing, mixing, shaping, expanding, partial dehydration, texture alteration, and the elimination of microorganisms or other toxic compounds. This process also involves thermal treatment and protein denaturation (Nwabueze and Iwe 2010; Chen et al. 2011).

Extrusion cooking offers essential advantages, including easy operation, continuous production, low labor requirements, cost-effectiveness, and limited waste generation, making it a versatile method for various applications. Notably, extrusion

can be employed as a pretreatment for the extraction of collagen from fish by-products. The Extrusion-Hydro-Extraction (EHE) technique intends to enhance the solubility of collagen chains in the sample through extrusion pretreatment. Huang et al. (2016) observed that high pressures reached during extrusion of scales from tilapia resulted in collagen yields between 7.5% and 12.3%, which is 2–3 times higher than yields from a normal AcOH extraction procedure.

Alternatively, some studies have explored sonication pretreatment to increase the extractability of collagen peptides. Ultrasounds bring cavitation in the liquid solvent, forming microbubbles that damage tissues of fish and increase the interaction area between liquid and solid. The shear forces produced by cavitation bubbles are influenced by the ultrasonic frequency. Zou et al. (2017) found that the yield of collagen extracted from soft-shelled turtle calipash increased by 16.3% with ultrasound pretreatment (24 min, 200 W, and 24 kHz sonication) compared to conventional AcOH extractions. Both extrusion and sonication techniques positively impact collagen yield and merit further investigation in future research (Zou et al. 2020; Ojha et al. 2020; Wang and Bao 2017).

Collagen Characterization Methods

In general, a comprehensive understanding and classification of collagen extracted from marine species are crucial for beginning a meaningful relationship between experimental results and structural features. This knowledge plays a key role in enabling researchers to manipulate results by altering the structure (Abraham et al. 2008). Various techniques can be employed to characterize marine collagen, whether in solution or solid-state, addressing chemical, structural, and morphological properties.

Chemical Composition of Collagen

Fourier transform infrared (FTIR) is a valuable tool for evaluating and identifying the presence, chemical composition, and type of collagen. Additionally, it serves as a means to compare collagen compositions extracted through different methods or examine the impact of isolation techniques on collagen composition (Kan et al. 2017; Jeevithan et al. 2014; Chuaychan et al. 2015). For instance, Chuaychan et al. (2015) utilized FTIR spectra to analyze collagens extracted via acid and pepsin-aided AcOH extraction procedures from seabass scales. The FTIR spectra indicated that the collagen analyzed corresponds to type I and reflected the treatments applied, with no discernible impact on functional groups due to the extraction process. However, variations in the width and height of corresponding signals in different graphs were observed, likely attributable to varying experimental conditions across studies, such as different solvents and concentrations (Kan et al. 2017; Jeevithan et al. 2014; Chuaychan et al. 2015).

Characterized Purity of Collagen and Breakdown

Electrophoresis techniques, such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), are commonly utilized to analyze protein patterns and determine the molecular weight distribution of collagen peptides. SDS-PAGE

allows for the separation of proteins and their fragments based on size, with larger chains being retained in the gel while smaller fragments migrate through the gel matrix (Silva et al. 2014). Comparing collagen bands obtained from various sources to a reference enables the determination of collagen types, and identification of amino acid sequences within the same collagen type is achievable (Silva et al. 2014; Fernandes-Silva et al. 2012).

Collagen, composed of three alpha chains, can have either identical or different alpha chains depending on the collagen type. For example, type I collagen comprises two alpha 1 chains and one alpha 2 chain, whereas type II collagen consists of three alpha 1 chains (Bielajew et al. 2020). Additionally, SDS-PAGE may reveal dimers (β-chains) or trimers (γ-chains) based on their assembly and posttranslational modification. Fish collagen, generally composed of two alpha 1 chains and one alpha 2 chain (approximately 100 kDa), has been observed in different discarded fish species such as rabbitfish, cuckoo ray, and common Atlantic grenadier, which exhibited variations in dimer and trimer components (Gelse et al. 2003). Rabbitfish, in particular, showed a weak peak for the beta component, and alpha 2 components were scarcely evident compared to other species.

Secondary Structure of Collagen

Circular dichroism (CD), based on the differential absorption of right and left circularly polarized light, is a valuable tool for assessing the secondary structure, binding, and folding characteristics of collagen, similar to other proteins (Polavarapu and He 2004; Greenfield 2006). CD analysis can distinguish between the native triple-helical structure and denatured coil structure of collagen, providing insights into its folding properties (Carvalho et al. 2018).

Collagen's supercoiled polyproline secondary structure (type II) exhibits well-defined CD transitions with positive and negative bands at 222 and 195 nm, respectively. Gelatin, as a denatured product of collagen, lacks characteristic CD signals, emphasizing that the CD signals of collagen primarily arise from the ordered fibril composed of triple-helical units (Drzewiecki et al. 2016). For proteins with slow folding processes, especially those with unknown folding properties like collagen, pre-folding is recommended before CD analysis, involving pre-folding the protein for several hours to days at 25 °C or on ice (Greenfield 2006).

The Hyp content in collagen influences its denaturation temperature, with higher Hyp content correlating with a higher denaturation temperature. For instance, collagen extracted with pepsin, exhibiting higher Hyp content, demonstrated a maximum denaturation temperature of 39.32 °C and a change in enthalpy (ΔH) of 0.91 J/g, while collagen isolated with acid showed a denaturation temperature of 38.17 °C and ΔH of 0.72 J/g (Chuaychan et al. 2015). In another study on seabass (*Lates calcarifer*), the Hyp content in collagen extracted from swim bladder and skin was reported as 83 and 79 (residues/1000 residues), respectively. The swim bladder collagen had a maximum temperature of 35.02 °C and a higher ΔH (0.918 J/g) compared to skin collagen (33.33 °C and ΔH of 0.860 J/g) (Davoodi et al. 1998).

4.5.2 Extraction of Gelatin

Gelatin, a soluble protein derived from the controlled hydrolysis of fibrous and insoluble collagen, undergoes a warm-water extraction process to convert collagen into a soluble form (Fig. 4.3). This process involves heat treatment, which disrupts the hydrogen and covalent bonds in the collagen triple helix, leading to a transition from helix to coil and the formation of soluble gelatin.

Several functional properties are associated with collagen and gelatin. Gel strength, often measured by the Bloom value test, assesses the strength of the gel formed. Both collagen and gelatin exhibit a thermoreversible gelation process, but in opposite directions: collagen gels melt as the temperature decreases, while gelatin gels melt with an increase in temperature. The commercial value of gelatin is often determined by its Bloom value. Fish gelatin typically displays gel strengths ranging from 0 to 426 g, with warm-water fish gelatin reported to have higher gel strengths compared to cold-water fish gelatin. This property contributes to the diverse applications and commercial uses of gelatin in various industries.

The setting and melting points of gelatin serve as crucial indicators of the quality of gelatin preparations. Gelatin gels, being thermoreversible, begin to melt when the temperature surpasses a specific point known as the melting point, typically lower

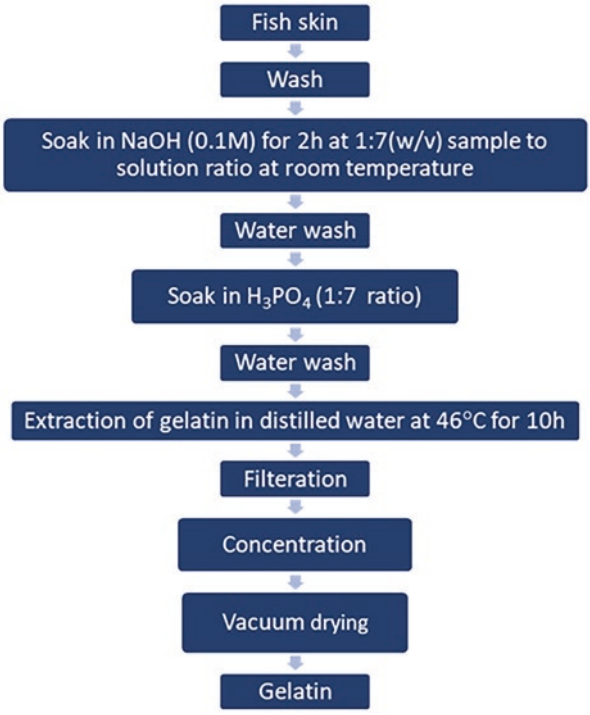

Fig. 4.3 General steps for isolation of gelatin from fish skin (Hanjabam et al. 2015)

than the human body temperature. For fish-derived gelatins, setting temperatures range from 8 to 25 °C, while melting temperatures range from 11 to 28 °C. The correlation between melting and gelling temperatures of gelatin and the proportion of proline and hydroxyproline in the original collagen has been demonstrated.

Unlike collagen, which is insoluble in water, fish type I collagen exhibits exceptional solubility in dilute acid compared to avian and mammalian collagen. Gelatin, although only partially soluble in cold water, undergoes swelling or hydration when stirred in water. Upon warming to around 40 °C, hydrated gelatin melts, forming a uniform solution. The solubility of collagen is influenced by the pH and NaCl concentration of the solution.

4.5.2.1 Collagen and Gelatin Hydrolysates (Hanjabam 2019)

Collagen and gelatin, despite possessing various functional properties, exhibit lower bioactivity due to their high molecular weight. To enhance bioactivities, collagen or gelatin hydrolysates are generated through controlled hydrolysis, employing methods such as acid, alkali, enzyme, or heat. This process breaks down peptide bonds, resulting in low-molecular-weight peptides with a typical range of 5.0–25 kDa. Gelatin hydrolysates can be produced using two approaches: enzymatic hydrolysis following gelatin extraction or direct preparation of hydrolysates without prior gelatin extraction, which can reduce processing time and costs.

Various proteases are commonly employed for hydrolysis, each with reported optimal conditions, including alcalase, pepsin, papain, trypsin, pancreatin, bromelain, flavourzyme, protamex, neutrase, and others. The comprehensive process for generating gelatin hydrolysate from fish skin, involving both thermal and enzymatic hydrolysis, is depicted in Fig. 4.4.

The hydrolysis of collagen or gelatin leads to the production of bioactive peptides that have potential applications in various processing industries, serving as natural preservatives. These peptides, originating from collagen and gelatin, demonstrate outstanding antioxidant properties and are being explored for diverse uses such as a plasticizer in protein films, antihypertensive agents, and cryoprotectants. This expands their roles beyond traditional antioxidant activities. The extraction of chemical components from seafood waste materials for application in different segments of the food industry represents a promising avenue for research and development in the utilization of seafood by-products.

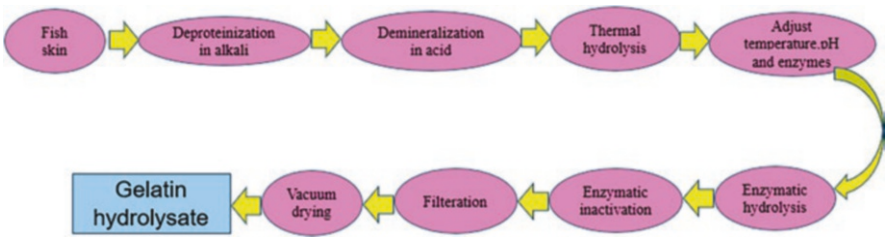

Fig. 4.4 Flow chart depicting enzymatic hydrolysis of fish skin gelatin (Hanjabam 2019)

4.6 Functions of Proteins

Marine biotechnology is an expanding field that integrates product chemistry, molecular biology, comparative biochemistry, and gene technology. The primary goal of marine biotechnology is the recovery and transformation of "value-added" products from marine organisms. Examples include obtaining specific surfactants, emulsifiers, pharmaceuticals, and flavorings through the treatment of fish protein with specific proteinases. Natural products derived from marine organisms, such as vaccines, enzymes, antifreeze proteins, antibiotics, hormones, and amino acids, exhibit various beneficial properties, including anti-proliferative, antimicrobial, anticoagulant, antihypertensive, and calcium-binding activities, contributing to improved health (Khan et al. 2020).

Protein plays a crucial role in constructing essential compounds like antibodies, enzymes, and hormones. It is also vital for the structural formation of tissues and cells in the body. The protein content serves as a significant energy source, providing 4 kcal/g. Infants, children, and pregnant women require dietary protein for depositing new protein in tissues and sustaining protein synthesis in adults' bodies. Inadequate protein intake can lead to diminished protein content in cells and organs, impairing their normal functions and increasing morbidity and mortality. However, excessive protein intake also has disadvantages (Young and Pellett 1987). Therefore, maintaining an appropriate level of protein content is crucial for ensuring a balanced and healthy long-term diet.

Protein calorie malnutrition is a significant cause of death in children in developing countries. Both adults and children who experience insufficient protein intake or struggle to convert this vital nutrient into energy for healthy tissue formation and proper organ function may develop protein calorie malnutrition. Prolonged inadequate protein intake can result in the loss of muscle mass, decreased immunity, and growth failure in affected individuals. Beyond their nutritional impact, fish proteins, such as gelatin and collagen, find applications (Fig. 4.5) in the cosmetic industry due to their excellent moisturizing properties, contributing to anti-aging and anti-wrinkle formulations.

Fish, with its diverse range of species, presents a more satiating effect compared to other animal protein sources and is often a more affordable option in tropical countries where various fish varieties are available.

4.7 Functions of Derivatives

4.7.1 Functions of Collagen

Collagen, the predominant structural protein in the extracellular matrix of various connective tissues, including skin, bones, ligaments, tendons, and cartilage, plays a crucial role in numerous biomedical applications. Its versatility has led to its use in biomaterials, such as tissue engineering, absorbable surgical sutures, osteogenic and bone-filling materials, hemostatic agents, immobilization of therapeutic

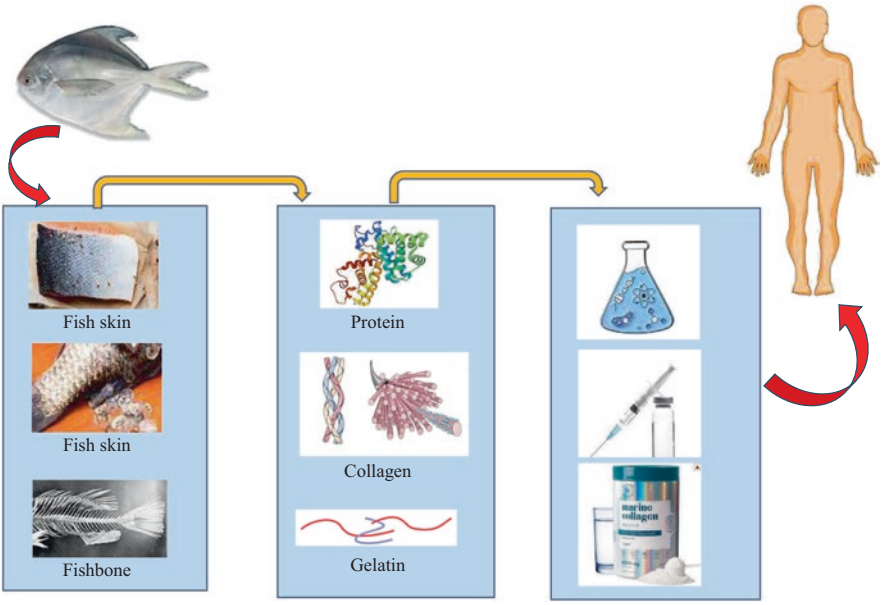

Fig. 4.5 Utilization of fish by-products (Alp-Erbay and Yeşilsu 2021)

enzymes, burn/wound cover dressings, and drug and gene carriers (Sahiner et al. 2014; Dong and Lv 2016; Xu et al. 2014; Wang et al. 2019; Ågren 2016; Silver 2009). In wound healing, collagen acts as a natural scaffold for new tissue growth, contributing to all phases of the healing process, including hemostasis, inflammation, proliferation, and remodeling (Silver 2009).

Endogenous collagen, produced by organisms, consists of three long helically shaped chains of amino acids, with the repeated sequence (Gly-X-Y)n forming collagen chains. Proline and 4-hydroxyproline (Hyp) commonly occupy the X and Y positions in the sequence (Davison-Kotler et al. 2019; Silva et al. 2014). Collagen degradation occurs with aging, exposure to ultraviolet light, and tobacco use, leading to issues such as dry skin, sagging skin, wrinkles, and stiff joints. Identifying new sources of collagen is essential for regenerative tissue applications (Silva et al. 2014; Aguda et al. 2014).

Exogenous collagen finds extensive use in food, biomaterials, and pharmaceutical applications. While mammalian collagen from cattle and pigs has been traditionally utilized, concerns such as bovine spongiform encephalopathy (BSE) outbreaks and religious constraints have limited its applications. In recent years, there has been a growing emphasis on sustainable development, and the concept of "Blue Growth" has gained traction, promoting sustainable growth in marine and maritime sectors as an environmentally friendly substitute for the sustainable use of natural resources (Kan et al. 2017; Mahboob 2015).

Caruso (2015) have highlighted that over 50% of fish tissues, including fins, heads, skin, and viscera, are often discarded as "waste," amounting to more than 20

million tons of by-products annually. This substantial volume of by-products from the seafood industry contains a wealth of valuable components. Notably, these by-products are rich in protein (10–25%) and lipid compounds (17–35%), making them an attractive resource (Silvipriya et al. 2015).

The utilization of marine by-products has become a significant focus, driven by factors such as easy extraction, high collagen content, low molecular weight facilitating absorption by the human body, biocompatibility, absence of risks associated with environmental friendliness, minimal biological contaminants and toxins, reduced regulatory and quality control challenges, fewer religious and ethical constraints, and animal diseases and pathogens (Shavandi et al. 2019a, b; Hou et al. 2016; Silvipriya et al. 2015). This attention to marine by-products as a source of collagen underscores their potential as a sustainable and valuable resource.

As a result, numerous studies have concentrated on the extraction and characterization of collagen from the skins of various fish species, including common Atlantic grenadier (*Nezumia aequalis*), catshark (*Galeus* spp.), small-spotted catshark (*Scyliorhinus canicula*), cod (*Gadus morhua*), rabbitfish (*Chimaera monstruosa*), lantern shark (*Etmopterus* spp.), cuckoo ray (*Leucoraja naevus*), and the scales and fins of *Cirrhinus mrigala* and *Catla catla* (Mahboob 2015; Blanco et al. 2019; Sotelo et al. 2016; Skierka and Sadowska 2007). Fish collagen from fins, skin, and bones typically exhibits a low denaturation temperature (25–30 °C for most fish species), contrasting with mammalian collagen (39–40 °C), and its variable composition poses challenges for biomedical applications (Rodríguez et al. 2017; Raman and Gopakumar 2018). The lower amino acid content, particularly proline and Hyp, contributes to the low denaturation temperature, making fish collagen less suitable for certain applications as it denatures at human body temperature. Current research is also exploring collagen from marine invertebrate organisms, including jellyfish and marine sponges (Khong et al. 2018; Tziveleka et al. 2017; Wu et al. 2019a, b).

The primary functions of collagen fibers in vertebrate tissues include preventing premature mechanical malfunction and aiding in storing, dissipating, and transmitting energy from musculoskeletal or externally applied forces. Collagen fibers provide crucial structural support to all body organs, ensuring firmness, elasticity, and strength required for repair through mechanochemical transduction processes, tissue regeneration, and effective locomotion (Tziveleka et al. 2017). Collagen actively contributes to the construction of a fibrous network of cells, known as fibroblasts, serving as the foundation for new cell growth. In areas like the dermis, collagen plays a protective role by inhibiting the absorption and spreading of pathogenic substances, environmental toxins, microorganisms, and cancerous cells (Shoulders and Raines 2009; Wu et al. 2019a, b). Several reviews are available on collagen resources for biomaterial development (Davison-Kotler et al. 2019; Lim et al. 2019), hydrolyzed collagen, and the sources and applications of marine collagen (Silva et al. 2014; Coppola et al. 2020).

4.7.2 Functional Properties of Fish Protein Hydrolysates

Functional properties refer to the overall physicochemical characteristics of proteins throughout the processing, storage, and consumption within a food system. Enzymatic hydrolysis, a process during which native proteins undergo changes, leads to the production of free amino acids and peptides. The valuable constituents of fish waste products can be recovered through the enzymatic hydrolysis method. Fish protein hydrolysates (FPHs) exhibit exceptional solubility across a broad range of ionic strengths and pH levels, and they generally withstand heat without precipitation. Additionally, when incorporated into food, FPHs have been observed to significantly enhance texture, emulsification, and water-holding capacity (Taheri et al. 2013). The functional attributes of fish protein and FPHs, including solubility, foaming properties, emulsifying properties, water-holding capacity, and fat-binding capacity, are detailed below.

4.7.2.1 Solubility

Solubility stands out as a crucial functional characteristic of protein hydrolysates (PHs), exerting a significant impact on other beneficial properties such as emulsification and foaming (De et al. 2020). The solubility of PHs is notably higher (>40%) than that of natural proteins, primarily attributed to the generation of small peptides resulting from protein breakdown (He et al. 2013). Hydrophobic and ionic interactions play a substantial role in protein solubility, with hydrophobic interactions diminishing solubility by promoting protein–protein interactions, while ionic interactions can enhance solubility by supporting protein–water interactions. The extent of protein solubility is influenced by the balance between hydrophobic and electrostatic interactions, where an increase in electrostatic repulsion compared to hydrophobic interactions leads to enhanced protein–solvent interactions (Bauer et al. 2017).

pH is another critical factor exerting a significant influence on solubility, with the lowest solubility observed at a pH of 6, corresponding to the isoelectric point. Enzymatic treatment emerges as a method to increase solubility, as it has the capacity to release medium or small peptides, exposing polar groups (Vijaykrishnaraj and Prabhasankar 2015)

4.7.2.2 Emulsifying Property

Emulsification capacity (EC) underscores the significance of utilizing proteins in emulsion-based food products due to their amphipathic structure, which facilitates absorption at the oil–water interface. Proteins are recognized as excellent emulsifiers, and their emulsion capacity relies on their ability to absorb at the oil–water interface. Emulsifiers, upon absorption, can safeguard dispersed segment particles from coalescence by forming thin layers at the oil–water interface or by reducing interfacial tension (Shevkani et al. 2015; Panpipat and Chaijan 2017).

The notable emulsion capacity of fish protein hydrolysates (FPHs) is primarily attributed to the partial unfolding and denaturation of proteins during the pH-shifting process. This process leads to the rapid absorption of the relatively

hydrophobic spherical head of the pH-treated protein onto non-polar lipid droplets (Kristinsson and Hultin 2003). The loss in tertiary structure, rather than secondary structure, can induce conformational changes at the oil–water interface. The pH-shifting process generates refolded proteins that outperform native proteins as they exhibit extended tertiary assembly.

4.7.2.3 Film-Forming Property

In recent times, there has been an increasing concern among researchers regarding environmental issues stemming from the widespread use of non-biodegradable materials in artificial packaging. This has led to the development of biodegradable packaging materials using biopolymers, with a particular focus on their biocompatibility and non-toxic properties. Polysaccharides, proteins, and lipids are extensively employed for developing biodegradable films, with proteins being a preferred choice due to their satisfactory mechanical and gas barrier properties and relative abundance. Among proteins, myofibrillar protein derived from fish muscle is utilized as a covering material. These muscle proteins exhibit solubility in pH solutions and insolubility in water.

Suspensions comprising protein, plasticizers, and solvents are employed to develop protein-based films. Various factors, such as the pH of the film-forming solution (FFS), plasticizers, preparation conditions, protein sources and concentration, and materials added to FFS, can influence the properties and formation of these films. The concentration of FFS plays a crucial role in the self-adhesion ability of polymers and the rate of polymer matrix formation in film suspensions. In a comparative study with polyvinyl chloride (PVC) film, it was found that a 3% protein content is effective in preparing blue marlin films. Color, an important indicator for customer acceptance in packaging applications, was evaluated by comparing the lightness, blueness/yellowness, and greenness/redness values of FMP films with different concentrations to PVC films. The study revealed that the concentration of FMP significantly influences the color of the films (Khan et al. 2020).

4.7.2.4 Foaming Property

The suitability and potential of proteins for specific food applications, such as whipped toppings, ice cream mixes, and baked goods, can be easily determined through their foaming capability and foaming capacity (Lam et al. 2018). Foam capacity is influenced by the pH of the protein solution, exhibiting minimum solubility at the isoelectric point. As the pH deviates from the isoelectric region toward either the acidic or alkaline side, the foaming capacity increases. However, a more significant increase is observed when moving toward alkaline pH compared to acidic pH (Yang and Baldwin 2017).

The stability of foams is dependent on the stability of the protein film formed in the gas–liquid interfacial layers and its absorbency capacity (Qin et al. 2013). Using better foam stabilizers can enhance protein-foam stability by increasing the thickness and facilitating the formation of layer-by-layer protein films at the interface (Kristinsson and Hultin 2003). Moreover, two key steps involved in foam formation

are the diffusion of solubilized proteins and their adsorption to the gas–liquid interfaces.

4.7.2.5 Gelling Property

Fish muscles are rich in proteins with high nutritional value, containing essential amino acids in maximum amounts. Collagen, a leading essential protein in animals, is abundantly present in fish bones and skin, which constitute approximately 75% of the total fish mass after filleting. These by-products are extensively utilized for various purposes. Collagen, characterized by high levels of hydroxyproline, glycine, and proline, can be denatured in dilute acid and converted into soluble protein by dissolving in a hot solution (Ahmad et al. 2017).

Heat-denatured collagen yields gelatin, which finds applications in the food, cosmetic, and pharmaceutical industries as an additive (stabilizer) to enhance resistance and reliability due to its unique properties. Gelatin is classified into two types based on the production process: type A, extracted through acid, and type B, extracted through alkali. Although they have different physicochemical effects, both types are used in various manufacturing sectors. While pig skin and cowhide are primary sources of commercial gelatin, there is an increasing demand for alternative sources, driven by religious or cultural considerations (Sha et al. 2014).

Fish gelatin, similar to mixed protein-polysaccharide and polysaccharides, can be used to produce edible films with thermoreversible gels. Fish gelatin is considered a promising alternative due to its functional edible properties and similarity to animal gelatin. Given religious restrictions and economic advantages, there is a growing interest in the fish processing industry to produce gelatin from fish waste (Khan et al. 2020).

4.7.2.6 Water-Holding Property

Water-holding capacity (WHC), the ability of food products to retain intrinsic water during processing, is a crucial attribute in the food industry. Products with high WHC are highly valued, and myofibrillar protein plays a significant role in this capacity. However, denaturation of myofibrillar protein can lead to a decrease in WHC. Trehalose, during frozen storage, helps maintain the three-dimensional structure of protein by interacting with water molecules through hydrogen bonds, preventing protein aggregation and preserving WHC.

Under frozen conditions, protein molecules lose their hydrophilic properties due to aggregation, reducing the ability of myofibrillar protein to form hydrogen bonds with water and resulting in decreased WHC. Additionally, cryoprotectants (CPA) can enhance WHC by increasing protein surface tension, allowing muscle tissues to hold more water. The interaction between water molecules and food materials is crucial for flavor retention, texture, mouthfeel, and WHC in food systems. Carbohydrate concentration also significantly influences WHC, with fish protein isolates (FPIs) exhibiting high water and oil holding capacities, enhancing their functional properties in various processed foods such as mayonnaise, baked products, and sausages.

For protein hydrolysates (PHs), water-holding capacity is a key hydrodynamic property, and fish protein hydrolysates (FPHs) are known for exceptional WHC due to increased concentrations of polar groups like carboxyl (COOH) and amino (NH_2) groups resulting from hydrolysis. FPHs exhibit significantly higher WHC compared to hydrolysates obtained from amino acid composition and fish protein. The abundance of aspartic acid over glutamic acid in FPH indicates a higher presence of hydrophilic groups, favoring water absorption (Vijaykrishnaraj and Prabhasankar 2015; Taheri et al. 2013).

4.7.2.7 Oil-Holding Property

The property or capacity to retain oil reflects a protein's ability to absorb and hold oil. This characteristic is closely tied to enzyme-substrate specificity and emulsifying capacity, influenced by factors such as bulk density and the degree of hydrolysis. In various food products, particularly in meat and confectionery, oil-holding capacity plays a significant role as it can impact the final product's taste (Taheri et al. 2013). The physical parameters of oil and the hydrophobicity of raw materials contribute to this property. Hydrolysis breaks down the protein chain, enhancing the hydrophobicity of fish protein products.

The non-polar residue of the protein is the primary factor responsible for hydrophobic interaction at the oil–water interface. Additionally, electrostatic, covalent, and hydrogen bonds may play a role in analyzing protein–lipid interactions. Blue wing sea robin fish protein hydrolysate (FPH) experienced a reduction in lipid holding volume and an increase in molecular weight due to its peptides. The oil-binding capacity of red salmon fish protein hydrolysate increased within a specific time range, suggesting that if hydrolysis were to extend further, the values would eventually decrease to an extreme oil-holding capacity of 7.8 ml oil/g. Palatase-confirmed protein, subjected to hydrolysis for 50 min at an E/S ratio of 0.5% at 50 °C, showed a decline in protein content (4.3 ml of oil/g) when the hydrolysis time was extended to 75 min. Similar results were observed in grass carp hydrolysates. Excessive hydrolysis compromises the stability of the protein structure, leading to the degradation of protein networks formed by entrapped oil.

The oil-holding capacity of many fish species surpasses that of commonly used food-grade oil binders, such as milk protein powder, soy protein powder, and casein powder. These fish proteins exhibit potential for use as industrial oil binders in food processing (Khan et al. 2020).

4.7.3 Marine Collagen Biomaterials Applications

Marine collagen, due to its water solubility, safety, biocompatibility, biodegradability, and easy extractability, has garnered attention for biomaterial applications. The abundance of marine waste by-products, such as fish skins, bones, scales, cartilage, and heads, has led to the utilization of marine-based collagen in diverse biomaterial applications, including:

Bone Tissue Engineering: Marine collagen has been employed in scaffolds for bone tissue engineering. Studies have investigated the osteogenic activity, mechanical properties, biocompatibility, and osteogenesis of these scaffolds. Collagen-hydroxyapatite scaffolds, for instance, have demonstrated stiffness, water bonding, and biodegradation characteristics suitable for bone tissue engineering.

Skin Tissue Engineering and Regeneration: Collagen derived from marine species, including jellyfish collagen, has been used for skin tissue engineering. It has been shown to promote adhesion, proliferation, and differentiation of human keratinocytes, facilitating skin regeneration.

Cartilage Tissue Engineering: Collagen fibrils derived from fish collagen have been developed for cartilage regeneration. Hydrogels based on fish collagen have shown promise as artificial cartilage, exhibiting excellent mechanical properties and biomechanical performance.

Wound Dressing: Marine collagen has been utilized in wound dressing applications. Fish collagen peptides have demonstrated wound-healing activity, enhancing the wound-healing process by protecting against infection and accelerating the three stages of healing.

Other Tissue Engineering Areas: Marine collagen has found applications in dental, vascular, corneal tissue engineering, and drug delivery systems. It has been incorporated into scaffolds for blood and lymphatic vessels, contributing to favorable interactions with surrounding tissues. Fish collagen has also been explored for drug delivery, with pH-sensitive hydrogels showing potential in improving drug bioavailability.

The versatility of marine collagen in various biomaterial applications highlights its potential as a sustainable and valuable resource in the field of regenerative medicine and tissue engineering.

4.7.4 Application of Fish Protein Hydrolysates

Fish protein hydrolysates have been promoted as health complements in developed countries. These foodstuffs, beyond providing nutritional benefits, have demonstrated specific health roles. The peptides present in FPH have shown various bioactive properties, including antioxidant, anti-obesity, immune modulation, anticoagulation, antimicrobial, anticancer, and antihypertensive effects.

References

Abraham LC, Zuena E, Perez-Ramirez B, Kaplan DL (2008) Guide to collagen characterization for biomaterial studies. J Biomed Mater Res Part B Appl Biomater 87:264–285. https://doi.org/10.1002/jbm.b.31078

Ågren M (2016) Wound healing biomaterials-volume 2: functional biomaterials. Woodhead Publishing, Sawston

Aguda AH, Panwar P, Du X, Nguyen NT, Brayer GD, Brömme D (2014) Structural basis of collagen fiber degradation by cathepsin K. Proc Natl Acad Sci USA 111:17474–17479. https://doi.org/10.1073/pnas.1414126111

Ahmad T, Ismail A, Ahmad SA, Khalil KA, Kumar Y, Adeyemi KD, Sazili AQ (2017) Recent advances on the role of process variables affecting gelatin yield and characteristics with special reference to enzymatic extraction: a review. Food Hydrocoll 63:85–96. https://doi.org/10.1016/j.foodhyd.2016.08.007

Ahmed R, Getachew AT, Cho Y-J, Chun B-S (2018) Application of bacterial collagenolytic proteases for the extraction of type I collagen from the skin of bigeye tuna (Thunnus obesus). LWT 89:44–51. https://doi.org/10.1016/j.lwt.2017.10.024

Alemán A, Martínez-Alvarez O (2013) Marine collagen as a source of bioactive molecules: a review. Nat Prod J 3:105–114. https://doi.org/10.2174/2210315511303020005

Alp-Erbay E, Yeşilsu AF (2021) Fish protein and its derivatives: functionality, biotechnology and health effects. Aquat Food Stud 1(1):AFS13. https://doi.org/10.4194/AFS13

Arumugam GKS, Sharma D, Balakrishnan RM, Ettiyappan JBP (2018) Extraction, optimization and characterization of collagen from sole fish skin. Sustain Chem Pharm 9:19–26. https://doi.org/10.1016/j.scp.2018.04.003

Askarieh G, Hedhammar M, Nordling K, Saenz A, Casals C, Rising A et al (2010) Self-assembly of spider silk proteins is controlled by a pH-sensitive relay. Nature 465:236–238

Bai C, Wei Q, Ren X (2017) Selective extraction of collagen peptides with high purity from cod skins by deep eutectic solvents. ACS Sustain Chem Eng 5:7220–7227. https://doi.org/10.1021/acssuschemeng.7b01439

Bauer KC, Haemmerling F, Kittelmann J, Duerr C, Goerlich F, Hubbuch J (2017) Influence of structure properties on protein–protein interactions—QSAR modeling of changes in diffusion coefficients. Biotechnol Bioeng 114(4):821–831. https://doi.org/10.1002/bit.26210

Bielajew BJ, Hu JC, Athanasiou KA (2020) Collagen: quantification, biomechanics and role of minor subtypes in cartilage. Nat Rev Mater 5:730–747. https://doi.org/10.1038/s41578-020-0213-1

Blanco M, Vázquez JA, Pérez-Martín RI, Sotelo CG (2017) Hydrolysates of fish skin collagen: an opportunity for valorizing fish industry byproducts. Mar Drugs 15:131. https://doi.org/10.3390/md15050131

Blanco M, Vázquez JA, Pérez-Martín RI, Sotelo CG (2019) Collagen extraction optimization from the skin of the small-spotted catshark (S. canicula) by response surface methodology. Mar Drugs 17:40. https://doi.org/10.3390/md17010040

Brazee SL, Carrington E (2006) Interspecific comparison of the mechanical properties of mussel byssus. Biol Bull 211:263–274. https://doi.org/10.2307/4134548

Burghagen A (1999) Collagen. In: Belitz HD, Grosch W (eds) Food chemistry, 2nd edn. Springer, Berlin, pp 540–547

Caruso G (2015) Fishery wastes and by-products: a resource to be valorised. J Fish Sci 9:80–83

Carvalho AM, Marques AP, Silva TH, Reis RL (2018) Evaluation of the potential of collagen from codfish skin as a biomaterial for biomedical applications. Mar Drugs 16:495. https://doi.org/10.3390/md16120495

Chalamaiah M, Hemalatha R, Jyothirmayi T (2012) Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. Food Chem 135(4):3020–3038

Chen W-H, Xu Y-Y, Hwang W-S, Wang J-B (2011) Pretreatment of rice straw using an extrusion/extraction process at bench-scale for producing cellulosic ethanol. Bioresour Technol 102:10451–10458. https://doi.org/10.1016/j.biortech.2011.08.118

Chen J, Li L, Yi R, Xu N, Gao R, Hong B (2016a) Extraction and characterization of acid-soluble collagen from scales and skin of tilapia (Oreochromis niloticus). LWT-Food Sci Technol 66:453–459. https://doi.org/10.1016/j.lwt.2015.10.070

Chen Y, Dan N, Wang L, Liu X, Dan W (2016b) Study on the cross-linking effect of a natural derived oxidized chitosan oligosaccharide on the porcine acellular dermal matrix. RSC Adv 6:38052–38063. https://doi.org/10.1039/C6RA03434A

Chinh NT, Manh VQ, Trung VQ, Lam TD, Huynh MD, Tung NQ, Trinh ND, Hoang T (2019) Characterization of collagen derived from tropical freshwater carp fish scale wastes and its amino acid sequence. Nat Prod Commun 14:1–12. https://doi.org/10.1177/1934578X19866288

Chuaychan S, Benjakul S, Kishimura H (2015) Characteristics of acid-and pepsin-soluble collagens from scale of seabass (Lates calcarifer). LWT Food Sci Technol 63:71–76. https://doi.org/10.1016/j.lwt.2015.03.002

Coppola D, Oliviero M, Vitale GA, Lauritano C, D'Ambra I, Iannace S, de Pascale D (2020) Marine collagen from alternative and sustainable sources: extraction, processing and applications. Mar Drugs 18(4):214. https://doi.org/10.3390/md18040214

Cumming MH, Hall B, Hofman K (2019) Isolation and characterisation of major and minor collagens from hyaline cartilage of Hoki (Macruronus novaezelandiae). Mar Drugs 17:223. https://doi.org/10.3390/md17040223

Davison-Kotler E, Marshall WS, García-Gareta E (2019) Sources of collagen for biomaterials in skin wound healing. Bioengineering 6:56. https://doi.org/10.3390/bioengineering6030056

Davoodi J, Wakarchuk WW, Surewicz WK, Carey PR (1998) Scan-rate dependence in protein calorimetry: the reversible transitions of Bacillus circulans xylanase and a disulfide-bridge mutant. Protein Sci 7:1538–1544. https://doi.org/10.1002/pro.5560070707

De D, Sandeep K, Kumar S, Raja RA, Mahalakshmi P, Sivaramakrishnan T, Vijayan K (2020) Effect of fish waste hydrolysate on growth, survival, health of Penaeus vannamei and plankton diversity in culture systems. Aquaculture 524:735240. https://doi.org/10.1016/j.aquaculture.2020.735240

Dong C, Lv Y (2016) Application of collagen scaffold in tissue engineering: recent advances and new perspectives. Polymers 8:42. https://doi.org/10.3390/polym8020042

Draget K, Philips G, Williams P (2000) Handbook of hydrocolloids. Woodhead Publishing, Cambridge

Drzewiecki KE, Grisham DR, Parmar AS, Nanda V, Shreiber DI (2016) Circular dichroism spectroscopy of collagen fibrillogenesis: a new use for an old technique. Biophys J 111:2377–2386. https://doi.org/10.1016/j.bpj.2016.10.023

Duan R, Zhang J, Du X, Yao X, Konno K (2009) Properties of collagen from skin, scale and bone of carp (Cyprinus carpio). Food Chem 112:702–706. https://doi.org/10.1016/j.foodchem.2008.06.020

Elavarasan K, Naveen Kumar V, Shamasundar BA (2014) Antioxidant and functional properties of fish protein hydrolysates from fresh water carp (Catla catla) as influenced by the nature of enzyme. J Food Process Preserv 38(3):1207–1214

Elavarasan K, Shamasundar BA, Badii F, Howell N (2016) Angiotensin I-converting enzyme (ACE) inhibitory activity and structural properties of oven-and freeze-dried protein hydrolysate from fresh water fish (Cirrhinus mrigala). Food Chem 206:210–216

Fernandes-Silva S, Moreira-Silva J, Silva S, Perez-Martin R, Sotelo C, Mano J, Marques A, Silva T, Reis R (2012) Marine collagen scaffolds crosslinked 'in situ' with genipin for cartilage regeneration. J Tissue Eng Regen Med 6:163

Gajanan PG, Elavarasan K, Shamasundar BA (2016) Bioactive and functional properties of protein hydrolysates from fish frame processing waste using plant proteases. Environ Sci Pollut Res 23(24):24901–24911

Gauza-Włodarczyk M, Kubisz L, Mielcarek S, Włodarczyk D (2017) Comparison of thermal properties of fish collagen and bovine collagen in the temperature range 298–670 K. Mater Sci Eng C 80:468–471. https://doi.org/10.1016/j.msec.2017.06.012

Gelse K, Pöschl E, Aigner T (2003) Collagens—structure, function, and biosynthesis. Adv Drug Deliv Rev Adv Drug Deliv Rev 55:1531–1546. https://doi.org/10.1016/j.addr.2003.08.002

Gildberg AR, Overbø K (1990) Purification and characterization of pancreatic elastase from Atlantic cod (Gadus morhua). Comp Biochem Physiol B 97(4):775–782

Gòmez-Guillèn MC, Turnay J, Fernández-Diaz MD, Ulmo N, Lizarbe MA, Montero P (2002) Structural and physical properties of gelatin extracted from different marine species: a comparative study. Food Hydrocoll 16:25–34

Govindharaj M, Roopavath UK, Rath SN (2019) Valorization of discarded Marine Eel fish skin for collagen extraction as a 3D printable blue biomaterial for tissue engineering. J Clean Prod 230:412–419. https://doi.org/10.1016/j.jclepro.2019.05.082

Greenfield NJ (2006) Using circular dichroism spectra to estimate protein secondary structure. Nat Protoc 1:2876. https://doi.org/10.1038/nprot.2006.202

Haard NF (1992) Biochemistry and chemistry of color and color change in seafoods. In: Advances in seafood biochemistry. Wiley, Hoboken, pp 305–360

Hang F, Eisoldt L, Hardy JG, Vendrely C, Coles M, Scheibel T et al (2010) A conserved spider silk domain acts as a molecular switch that controls fibre assembly. Nature 465:239–242

Hanjabam MD (2019) Extraction of collagen, gelatin and its derivatives from fish wastes ITEC training programme. In: Protocols for the production of high value secondary products from industrial fish and shellfish processing. ICAR-CIFT, Kochi

Hanjabam MD, Kannaiyan SK, Kamei G, Jakhar JK, Chouksey MK, Gudipati V (2015) Optimisation of gelatin extraction from Unicorn leatherjacket (Aluterus monoceros) skin waste: response surface approach. J Food Sci Technol 52(2):976–983

He S, Franco C, Zhang W (2013) Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). Food Res Int 50(1):289–297

He G, Yin Y, Yan X, Wang Y (2017) Semi-bionic extraction of effective ingredient from fishbone by high intensity pulsed electric fields. J Food Process Eng 40:e12392. https://doi.org/10.1111/jfpe.12392

He G, Yan X, Wang X, Wang Y (2019) Extraction and structural characterization of collagen from fishbone by high intensity pulsed electric fields. J Food Process Eng 42:e13214. https://doi.org/10.1111/jfpe.13214

Hou Y, Shavandi A, Carne A, Bekhit AA, Ng TB, Cheung RCF, Bekhit AE-DA (2016) Marine shells: potential opportunities for extraction of functional and health-promoting materials. Crit Rev Environ Sci Technol 46:1047–1116. https://doi.org/10.1080/10643389.2016.1202669

Huang C-Y, Kuo J-M, Wu S-J, Tsai H-T (2016) Isolation and characterization of fish scale collagen from tilapia (Oreochromis sp.) by a novel extrusion–hydro-extraction process. Food Chem 190:997–1006. https://doi.org/10.1016/j.foodchem.2015.06.066

Huss HH (1995) Quality and quality changes in fresh fish. FAO, Rome, p 195

Ibáñez JAME, Castro-Puyana M (2016) Supercritical fluid extraction. In: Encyclopedia of food and health. Academic Press, Oxford, pp 227–233. https://doi.org/10.1016/B978-0-12-384947-2.00675-9

Jafari H, Lista A, Mafosso M, Siekapen, Ghaffari-Bohlouli P, Nie L, Alimoradi H, Shavandi A (2020) Fish collagen: extraction, characterization, and applications for biomaterials engineering. Polymers 12(10):2230. https://doi.org/10.3390/polym12102230

Jeevithan E, Bao B, Bu Y, Zhou Y, Zhao Q, Wu W (2014) Type II collagen and gelatin from silvertip shark (Carcharhinus albimarginatus) cartilage: isolation, purification, physicochemical and antioxidant properties. Mar Drugs 12:3852–3873. https://doi.org/10.3390/md12073852

Jongjareonrak A, Benjakul S, Visessanguan W, Nagai T, Tanaka M (2005) Isolation and characterisation of acid and pepsin-solubilised collagens from the skin of Brownstripe red snapper (Lutjanus vitta). Food Chem 93:475–484. https://doi.org/10.1016/j.foodchem.2004.10.026

Kan M, Qian X, Zhang T, Yue D, Zhao Y (2017) Highly active IrO x nanoparticles/black Si electrode for efficient water splitting with conformal TiO_2 interface engineering. ACS Sustain Chem Eng 5:10940–10946. https://doi.org/10.1021/acssuschemeng.7b02850

Khan SB, Qian Z-J, Ryu B, Kim S-K (2009) Isolation and biochemical characterization of collagens from seaweed pipefish, Syngnathus schlegeli. Biotechnol Bioprocess Eng 14:436–442. https://doi.org/10.1007/s12257-009-0007-1

Khan S, Rehman A, Shah H, Aadil RM, Ali A, Shehzad Q, Ashraf W, Yang F, Karim A, Khaliq A, Xia W (2020) Fish protein and its derivatives: the novel applications, bioactivities, and their functional significance in foods. Food Rev Intl. https://doi.org/10.1080/87559129.2020.1828452

Khong NM, Yusoff FM, Jamilah B, Basri M, Maznah I, Chan KW, Armania N, Nishikawa J (2018) Improved collagen extraction from jellyfish (Acromitus hardenbergi) with increased

physical-induced solubilization processes. Food Chem 251:41–50. https://doi.org/10.1016/j.foodchem.2017.12.083
Kimura S, Miyauchi Y, Uchida N (1991) Scale and bone type I collagens of carp (Cyprinus carpio). Comp Biochem Physiol B Comp Biochem 99:473. https://doi.org/10.1016/0305-0491(91)90073-M
Kittiphattanabawon P, Benjakul S, Visessanguan W, Shahidi F (2010) Isolation and characterization of collagen from the cartilages of brownbanded bamboo shark (Chiloscyllium punctatum) and blacktip shark (Carcharhinus limbatus). LWT-Food Sci Technol 43:792–800. https://doi.org/10.1016/j.lwt.2010.01.006
Kristinsson HG, Hultin HO (2003) Effect of low and high pH treatment on the functional properties of cod muscle proteins. J Agric Food Chem 51(17):5103–5110. https://doi.org/10.1021/jf026138d
Lam A, Can Karaca A, Tyler R, Nickerson M (2018) Pea protein isolates: structure, extraction, and functionality. Food Rev Int 34(2):126–147
Liang Q, Wang L, Sun W, Wang Z, Xu J, Ma H (2014) Isolation and characterization of collagen from the cartilage of Amur sturgeon (Acipenser schrenckii). Process Biochem 49:318–323. https://doi.org/10.1016/j.procbio.2013.12.003
Lim Y-S, Ok Y-J, Hwang S-Y, Kwak J-Y, Yoon S (2019) Marine collagen as a promising biomaterial for biomedical applications. Mar Drugs 17:467. https://doi.org/10.3390/md17080467
Liu H, Huang K (2016) Structural characteristics of extracted collagen from tilapia (Oreochromis mossambicus) bone: effects of ethylenediaminetetraacetic acid solution and hydrochloric acid treatment. Int J Food Prop 19:63–75. https://doi.org/10.1080/10942912.2014.951939
Liu D, Zhou P, Li T, Regenstein JM (2014) Comparison of acid-soluble collagens from the skins and scales of four carp species. Food Hydrocoll 41:290–297. https://doi.org/10.1016/j.foodhyd.2014.04.030
Lucas JM, Vaccaro E, Waite JH (2002) A molecular, morphometric and mechanical comparison of the structural elements of byssus from Mytilus edulis and Mytilus galloprovincialis. J Exp Biol 205:1807–1817
Luo Q-B, Chi C-F, Yang F, Zhao Y-Q, Wang B (2018) Physicochemical properties of acid-and pepsin-soluble collagens from the cartilage of Siberian sturgeon. Environ Sci Pollut Res 25:31427–31438. https://doi.org/10.1007/s11356-018-3147-z
Mahboob S (2015) Isolation and characterization of collagen from fish waste material-skin, scales and fins of Catla catla and Cirrhinus mrigala. J Food Sci Technol 52:4296–4305. https://doi.org/10.1007/s13197-014-1520-6
Meyer M (2019) Processing of collagen based biomaterials and the resulting materials properties. Biomed Eng Online 18:24. https://doi.org/10.1186/s12938-019-0647-0
Numata K (2020) How to define and study structural proteins as biopolymer materials. Polym J 52:1043–1056. https://doi.org/10.1038/s41428-020-0362-5
Nwabueze TU, Iwe MO (2010) Residence time distribution (RTD) in a single screw extrusion of African breadfruit mixtures. Food Bioprocess Technol 3:135. https://doi.org/10.1007/s11947-008-0056-z
Ojha KS, Aznar R, O'Donnell C, Tiwari BK (2020) Ultrasound technology for the extraction of biologically active molecules from plant, animal and marine sources. TrAC Trends Anal Chem 122:115663. https://doi.org/10.1016/j.trac.2019.115663
Otani H, Dong XY, Hosono A (1990) Preparation of low immunogenic peptide fragments from milk casein. Milchwissenschaft 45:217–220
Panpipat W, Chaijan M (2017) Functional properties of pH-shifted protein isolates from bigeye snapper (Priacanthus Tayenus) head by-product. Int J Food Prop 20(3):596–610. https://doi.org/10.1080/10942912.2016.1171778
Paul RG, Bailey AJ (2003) Chemical stabilisation of collagen as a biomimetic. Sci World J 3:138–155. https://doi.org/10.1100/tsw.2003.13
Polavarapu PL, He J (2004) Peer reviewed: chiral analysis using mid-IR vibrational CD spectroscopy. ACS Publications, Washington, pp 61–67. https://doi.org/10.1021/ac0415096

Prestes RC (2013) Colágeno e seus derivados: características e aplicações em produtos cárneos. J Health Sci 15:791. https://doi.org/10.17921/2447-8938.2013v15n1p%p
Qin X, Waite JH (1995) Exotic collagen gradients in the byssus of the mussel Mytilus edulis. J Exp Biol 198:633–644
Qin X, Coyne KJ, Waite JH (1997) Tough tendons mussel byssus has collagen with silk-like domains. J Biol Chem 272:32623–32627. https://doi.org/10.1074/jbc.272.51.32623
Qin Z, Guo X, Lin Y, Chen J, Liao X, Hu X, Wu J (2013) Effects of high hydrostatic pressure on physicochemical and functional properties of walnut (Juglans Regia L.) protein isolate. J Sci Food Agric 93(5):1105–1111. https://doi.org/10.1002/jsfa.5857
Raa, J. 1990. Biotechnology in aquaculture and the fish processing industry: a success story in Norway. In: M. N. Voigt and J. R. Botta (eds) Advances in fisheries technology and biotechnology for increased profitability. Lancaster: Technomic Publishing Co, p. 509-524.
Raman M, Gopakumar K (2018) Fish collagen and its applications in food and pharmaceutical industry: a review. EC Nutr 13:752–767
Ramli L, Natsir H, Dali S, Danial S (2019) Collagen extraction from bone of Lutjanus sp. and toxicity assay. J Akta Kim Indones 12:67–72. https://doi.org/10.20956/ica.v12i1.5925
Rodríguez F, Moran L, González G, Troncoso E, Zuñiga R (2017) Collagen extraction from mussel byssus: a new marine collagen source with physicochemical properties of industrial interest. J Food Sci Technol 54:1228–1238. https://doi.org/10.1007/s13197-017-2566-z
Sahiner M, Alpaslan D, Bitlisli BO (2014) Collagen-based hydrogel films as drug-delivery devices with antimicrobial properties. Polym Bull 71:3017–3033. https://doi.org/10.1007/s00289-014-1235-x
Schmidt M, Dornelles R, Mello R, Kubota E, Mazutti M, Kempka A, Demiate I (2016) Collagen extraction process. Int Food Res J 22:913–922
Sha X-M, Tu Z-C, Liu W, Wang H, Shi Y, Huang T, Man Z-Z (2014) Effect of ammonium sulfate fractional precipitation on gel strength and characteristics of gelatin from bighead carp (Hypophthalmichthys Nobilis) scale. Food Hydrocoll 36:173–180. https://doi.org/10.1016/j.foodhyd.2013.09.024
Shahidi F, Synowiecki J, Penney RW (1993) Pigmentation of Artic char (Salvelinus alpinus) by dietary carotenoids. J Aquat Food Prod Technol 2(1):99–115
Shalaby M, Agwa M, Saeed H, Khedr SM, Morsy O, El-Demellawy MA (2020) Fish scale collagen preparation, characterization and its application in wound healing. J Polym Environ 28:166–178. https://doi.org/10.1007/s10924-019-01594-w
Shavandi A, Hou Y, Carne A, McConnell M, Bekhit AE-DA (2019a) Chapter four-marine waste utilization as a source of functional and health compounds. In: Toldrá F (ed) Advances in food and nutrition research, vol 87. Academic, Cambridge, pp 187–254
Shavandi A, Saeedi P, Bekhit AE-D (2019b) Pulsed electric field processing. In: Ozogul Y (ed) Innovative technologies in seafood processing. CRC, Boca Raton, pp 155–174
Shevkani K, Singh N, Kaur A, Rana JC (2015) Structural and functional characterization of kidney bean and field pea protein isolates: a comparative study. Food Hydrocoll 43:679–689. https://doi.org/10.1016/j.foodhyd.2014.07.024
Shoulders MD, Raines RT (2009) Collagen structure and stability. Annu Rev Biochem 78:929–958. https://doi.org/10.1146/annurev.biochem.77.032207.120833
Silva TH, Moreira-Silva J, Marques AL, Domingues A, Bayon Y, Reis RL (2014) Marine origin collagens and its potential applications. Mar Drugs 12:5881–5901. https://doi.org/10.3390/md12125881
Silver FH (2009) The importance of collagen fibers in vertebrate biology. J Eng Fibers Fabr 4:9–17. https://doi.org/10.1177/155892500900400203
Silvipriya K, Kumar KK, Bhat A, Kumar BD, John A, Lakshmanan P (2015) Collagen: animal sources and biomedical application. J Appl Pharm Sci 5:123–127. https://doi.org/10.7324/JAPS.2015.50322
Simpson BK, Haard NF (1987) Cold-adapted enzymes from fish. In: Knorr D (ed) Food biotechnology. Marcel Dekker, New York, pp 495–527

Sionkowska A, Kozlowska J (2014) Fish scales as a biocomposite of collagen and calcium salts. In: Key engineering materials, vol 587. Trans Tech Publications Ltd., Stafa-Zurich, pp 185–190

Skierka E, Sadowska M (2007) The influence of different acids and pepsin on the extractability of collagen from the skin of Baltic cod (Gadus morhua). Food Chem 105:1302–1306. https://doi.org/10.1016/j.foodchem.2007.04.030

Sotelo CG, Comesaña MB, Ariza PR, Pérez-Martín RI (2016) Characterization of collagen from different discarded fish species of the west coast of the Iberian Peninsula. J Aquat Food Prod Technol 25:388–399. https://doi.org/10.1080/10498850.2013.865283

Sousa RO, Alves AL, Carvalho DN, Martins E, Oliveira C, Silva TH, Reis RL (2020a) Acid and enzymatic extraction of collagen from Atlantic cod (Gadus Morhua) swim bladders envisaging health-related applications. J Biomater Sci Polym Ed 31:20–37. https://doi.org/10.1080/09205063.2019.1669313

Sousa RO, Martins E, Carvalho DN, Alves AL, Oliveira C, Duarte ARC, Silva TH, Reis RL (2020b) Collagen from Atlantic cod (Gadus morhua) skins extracted using CO_2 acidified water with potential application in healthcare. J Polym Res 27:1–9. https://doi.org/10.1007/s10965-020-02048-x

Taheri A, Anvar S, Ahari H, Fogliano V (2013) Comparison the functional properties of protein hydrolysates from poultry by-products and rainbow trout (Onchorhynchus Mykiss) viscera. Iran J Fish Sci 12(1):154–169

Tan Y, Chang SK (2018) Isolation and characterization of collagen extracted from channel catfish (Ictalurus punctatus) skin. Food Chem 242:147–155. https://doi.org/10.1016/j.foodchem.2017.09.013

Tsuchiya K, Numata K (2017) Chemical synthesis of multiblock copolypeptides inspired by spider dragline silk proteins. ACS Macro Lett 6:103–106

Tziveleka L-A, Ioannou E, Tsiourvas D, Berillis P, Foufa E, Roussis V (2017) Collagen from the marine sponges Axinella cannabina and Suberites carnosus: isolation and morphological, biochemical, and biophysical characterization. Mar Drugs 15:152. https://doi.org/10.3390/md15060152

Van De Water L, Varney S, Tomasek JJ (2013) Mechanoregulation of the myofibroblast in wound contraction, scarring, and fibrosis: opportunities for new therapeutic intervention. Adv Wound Care 2:122–141. https://doi.org/10.1089/wound.2012.0393

Veeruraj A, Arumugam M, Balasubramanian T (2013) Isolation and characterization of thermostable collagen from the marine eel-fish (Evenchelys macrura). Process Biochem 48:1592–1602. https://doi.org/10.1016/j.procbio.2013.07.011

Vijaykrishnaraj M, Prabhasankar P (2015) Marine protein hydrolysates: their present and future perspectives in food chemistry–a review. RSC Adv 5(44):34864–34877. https://doi.org/10.1039/C4RA17205A

Wang JM, Bao J-Q (2017) The ultrasonic-assisted solvent extraction and characterization of fishskin collagen. Acta Agric Shanghai 2:21

Wang E, Han J, Zhang X, Wu Y, Deng X-L (2019) Efficacy of a mineralized collagen bone-grafting material for peri-implant bone defect reconstruction in mini pigs. Regen Biomater 6:107–111. https://doi.org/10.1093/rb/rby029

Wijaya H, Putriani S, Safithri M, Tarman K (2020) Isolation and allergenicity of protein collagen from parang-parang fish skin (Cirocentrus dorab). IOP Conf Ser Earth Environ Sci 411:012054

Wu J, Guo X, Liu H, Chen L (2019a) Isolation and comparative study on the characterization of guanidine hydrochloride soluble collagen and pepsin soluble collagen from the body of surf clam shell (Coelomactra antiquata). Food Secur 8:11. https://doi.org/10.3390/foods8010011

Wu J, Kong L, Zhang J, Chen W (2019b) Extraction and properties of acid-soluble collagen and pepsin-soluble collagen from silver carp (Hypophthalmichthys molitrix) scales: prerequisite information for fishery processing waste reuse. Pol J Environ Stud 28:2923–2930. https://doi.org/10.15244/pjoes/93742

Xu H, Xu H, Zhang L, Qu X, Zhao B (2014) Absorbable collagen suture and non-absorbable silk suture in oral implantation. Chin J Tissue Eng Res 18:1877

Yang S-C, Baldwin RE (2017) Functional properties of eggs in foods. In: Egg science and technology. CRC Press, Boca Raton, pp 405–463

Yang H, Duan L, Li Q, Tian Z, Li G (2018) Experimental and modeling investigation on the rheological behavior of collagen solution as a function of acetic acid concentration. J Mech Behav Biomed Mater 77:125–134. https://doi.org/10.1016/j.jmbbm.2017.09.003

Yin S-J, Zhang L, Zhang L, Wan J, Song W, Jiang X, Park Y-D, Si Y-X (2018) Metabolic responses and arginine kinase expression of juvenile cuttlefish (Sepia pharaonis) under salinity stress. Int J Biol Macromol 113:881–888. https://doi.org/10.1016/j.ijbiomac.2018.03.036

Young VR, Pellett PL (1987) Protein intake and requirements with reference to diet and health. Am J Clin Nutr 45(5):1323–1343. https://doi.org/10.1093/ajcn/45.5.1323

Yunoki S, Suzuki T, Takai M (2003) Stabilization of low denaturation temperature collagen from fish by physical cross-linking methods. J Biosci Bioeng 96:575–577. https://doi.org/10.1016/S1389-1723(04)70152-8

Żelechowska E, Sadowska M, Turk M (2010) Isolation and some properties of collagen from the backbone of Baltic cod (Gadus morhua). Food Hydrocoll 24:325–329. https://doi.org/10.1016/j.foodhyd.2009.10.010

Zou Y, Wang L, Cai P, Li P, Zhang M, Sun Z, Sun C, Xu W, Wang D (2017) Effect of ultrasound assisted extraction on the physicochemical and functional properties of collagen from soft-shelled turtle calipash. Int J Biol Macromol 105:1602–1610. https://doi.org/10.1016/j.ijbiomac.2017.03.011

Zou Y, Yang H, Zhang X, Xu P, Jiang D, Zhang M, Xu W, Wang D (2020) Effect of ultrasound power on extraction kinetic model, and physicochemical and structural characteristics of collagen from chicken lung. Food Prod Process Nutr 2:3. https://doi.org/10.1186/s43014-019-0016-1

Bioinformatics and Computational Tools

5

Maya Raman

Abstract

Proteomics and bioinformatics are now considered as very important tools for the characterization and understanding of any food biomolecules. It has a huge significance in providing nutritional and livelihood security. Genomic research is also equally important. The existing high-throughput next-generation technologies generate oceanic data that require appropriate tools for extensive analysis. Gene-based information and computational technologies are integrated in bioinformatics to produce new data and knowledge for the benefits of food and health. In this chapter, different tools relevant to proteins and genes are discussed in details for the benefits of researchers, students and academicians. These include different bioinformatics tools, data banks and many more. The purpose of the chapter is to familiarize the concept and extend the practical translation to the relevant researchers and industry.

Keywords

Proteomics · Genomics · Bioinformatics · Proteins · Biology

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5.1 Introduction

The intersection of growing population, scarcity of sustainable resources and change in climate has created challenging obstacles for ensuring global food security. Concurrently, the rising consumer demand for safe, nutritious food has spotlighted the "*blue foods*," chiefly due to its rich nutritional profile and protein digestibility. Extensive research findings highlight the manifold potentialities of fish proteins including health promotion, disease prevention and therapeutic interventions. The comprehensive understanding of proteins is essential to harness the broad spectrum applications of fish proteins and stimulate the growth of the blue bioeconomy (Yuan et al. 2023). Owing to the growing demand, to increase the production in a sustainable manner, the aquaculture sector has been witnessing several challenges including safety, disease resistance and quality of aqua foods, trials with respect to climate change and so on. These are addressed using various proteomic techniques. Various proteomic techniques along with bioinformatics resources find uses in diverse areas of fisheries including safety, quality and health (Nissa et al. 2021a, b).

The past decade has heralded remarkable technological and scientific progresses and has generated comprehensive data sets particularly in the form of sequencing, microarray technologies and mass spectrometry for various biological systems. Nevertheless, the extensive knowledge is still vague and fails to give an understanding of how a system functions or behaves. The complexity of the system is reflected through its complex functions; and it demonstrates that the emergent function or behavior of a system arises from the totality of the system interactions that may be non-linear and stochastic (Nissa et al. 2021a, b). The advent of systems biology and computational modeling has sophisticated the understanding of system structure, functional dynamics and system behavior. Systems biology is a holistic process for appreciating the biological processes at cell, tissue or organism level. With this intention, omics technologies involve whole genome, transcriptome and proteome study. The history of the suffix *-ome* goes back to 1920 when the term *genome* was used first for "whole" genetic makeup of an organism hinting that suffix *-ome* refers to "*complete or whole*" (Yadav 2007). Subsequently, the suffix *-omics* was applicable for high-throughput and comprehensive study of genes (genomics), transcripts (transcriptomics), proteins (proteomics), etc. (Yadav 2007). The proteome is dynamic in nature and varies with tissue, physiological condition and environment. This provides information about the gene expression and post-transcriptional and post-translational protein expressions (Schwanhäusser et al. 2011). Consequently, in nutshell, it gives a snapshot of the organisms' state of being and records its adaptive potential. Recent reports demonstrate the application of proteomics in fisheries sector could be a powerful tool to provide valuable perception about the composition of the raw materials and quality changes during different stages of processing and storage (Piras et al. 2016). The nutritional sciences research is idyllic for the application of systems biology approaches. Further, with advances in proteome- and peptidome-*omics* of different species, protein databases are available for identification of proteins, gene ontology and phylogenetic assessments.

5.1.1 Systems Biology

The systems biology focuses on the fundamental network formation and dynamics of molecular interactions, which in turn are categorized as signaling, regulatory or metabolic interactions. Computational analyses of networks are characterized as qualitative or quantitative (Reed et al. 2006; Ulrich et al. 2008). The qualitative or structural network analyses emphasize chiefly on characteristics or properties derived using mathematical graph theory, while the quantitative analyses measure and model precise kinetic parameters. Nevertheless, acquiring precise kinetic parameters is difficult, and most of the *-omic* data sets are semi-quantitative and of limited order. Qualitative approaches are applied in biological networks and several lines of data emphasize network connectivity and yield precise prediction of network dynamics (Ruths et al. 2008; Komurov and Ram 2010). Metabolic networks significant for metabolic engineering are also build through an amalgamation of database mining and literature curation, which are modeled mathematically (in silico model predictions developed using constraint-based models) and iteratively validated using wet laboratory experiments (Becker et al. 2007). In recent years, these metabolic reconstructions have gained significant attention and have been successfully adopted to demonstrate the predictable potentialities of the novel antimicrobial targets (Shen et al. 2010).

Therefore, the high-throughput technologies have revolutionized the molecular biology system through genomics (genome of the organism), transcriptomics (set of RNA molecules), proteomics (protein structure and functions) and metabolomics (metabolites) (Fig. 5.1). Since proteins form the ground link between gene and mRNA, several molecular and cellular structure and organization, and the physiological and pathological processes are demonstrated using proteins. Henceforth, high-throughput proteomic techniques assist biologist and biomedical scientists to achieve an enhanced comprehension of basic molecular biology and disease process or bioactive potentialities of proteins (Decramer et al. 2006).

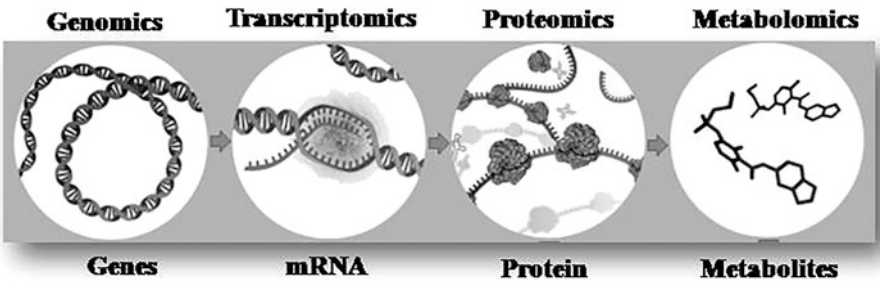

Fig. 5.1 Systems biology and different *-omics*

5.1.2 Proteomics

Proteome (term coined by Mark Wilkins in 1994) is the protein complement of genome signifying its end product. In other words, genome is a blueprint of the extensive and multifaceted network of interfaces facilitated by the proteins (Nissa et al. 2022). In an organism, the genome remains continual for all cells unlike proteome that is vibrant and varies from cell to cell in response to the changing stages and other extrinsic factors (Morro et al. 2020; Nissa et al. 2021a). Since proteins are the ultimate effectors of all biological activities within the system, it is as important as genomics (Nissa et al. 2021b). Though protein expression analysis is multifaceted, it is functionally useful. Among its extensive applications, proteome analyses can be utilized for marker proteins/peptides for adulteration detection and deceptive practices (Pampanin et al. 2014; Saleh et al. 2018). Ultimately, innovative methodologies that are accurate and reliable can be developed and confirmed for various applications (López et al. 2002; Ortea et al. 2020). Advances in genomics and proteomics have unlocked the access to gene expression studies on an exceptional scale.

The arena of proteomics was introduced based on *one gene-one enzyme* theory of Beadle and Tatum's (early 1940s). Proteomics is recognized as an essential disciple for the accurate building of network models, and it is inevitable in the systems biology (Weston and Hood 2004). The PubMed has shown a linear increase in the proteomic publications and data sets. The quantum of research associated with proteomics is almost three times that of systems biology. However, the systems biology and proteomic researches relevant to nutritional research are limited. Advances in scientific experimental techniques including mass spectrometry (electrospray ionization, matrix-assisted laser desorption ionization, tandem MS/MS) paved way to innovations in proteomics. Presently, MS-based proteomics is used for systemic characterization, identification and quantification. It also gives an idea about the protein interactions and post-translational modifications in proteins (Noor et al. 2021). The intact protein or fragments (peptides) are identified by bottom-up or top-down approach; nevertheless, the former is most favored for high-throughput analytical programs, as these are easy to handle and the physiological properties are uniform (Mohr et al. 2010). In the bottom-up method (peptide-based proteomics), the proteins are digested enzymatically (trypsin) and purified using column for peptide analysis by MS (MS1 spectra) (Pandey and Mann 2000). The efficiency of MS for the complex biological matrices is improved by employing highly efficient separation technologies such as 2-dimensional gel electrophoresis (2DGE), HPLC and LC-MS. The bottom-up approach is primarily compelled by the application of collision-induced dissociation fragmentation to peptide MS. The proteins digested and separated by LC for MS evaluation are also called "shotgun" proteomics. The latest advances in technologies such as electron capture dissociation and its association with high-resolution mass analyzers (Orbitrap) have supported the assessment of intact protein molecular mass, as in top-down approach (Macek et al. 2006). Top-down proteomics approach recognizes proteins of molecular mass >200 kDa (Han et al. 2006). These technologies have their limitations. The bottom-up approach has low protein sequence coverage,

while top-down approach is effected by the poor solubility of the proteins. SDS used for washing cannot be used in ESI (Dalmasso et al. 2009). Proteomics workflow comprises of sample preparation and analytical flow (separation, identification and validation). Numerous approaches are in practice for the quantitative analysis of peptides and proteins, as a part of systems biology approach. Basic strategy includes biomarker discovery, where relative quantification of proteins/peptides in samples is determined, rather than absolute amount, and these are compared for evaluation (untreated vs treated) (Pan et al. 2009). The global quantification strategy may be labeled (differential mass tagging or isotopic labeling) or label-free (Ong and Mann 2005). Accordingly, proteomics is classified into expression, functional and structural proteomics (Hanash 2003).

Expression proteomics: This is a unique method to study the qualitative and quantitative expression of proteins. The aim is to identify the difference in protein expressions between two conditions (treated and untreated) (Banks et al. 2000); identify either specific proteins and/or new signaling proteins (Graves and Haystead 2002) and compare the protein expression pattern in different cells (Chandrasekhar et al. 2014). The 2-DE and MS techniques can be utilized for this approach.

Structural proteomics: For the determination of 3D structure and structural complexities of functional proteins, nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography are used. This approach specifies the various interactions between proteins including membrane, cell organelles, ribosomes and nuclear protein (Rout et al. 2000; Jungbauer and Hahn 2009).

Functional proteomics: This type studies the protein functions and molecular mechanism in cells and determines the interactions between proteins and with other biomolecules (Monti et al. 2007).

5.1.2.1 Extraction, Separation and Isolation of Proteins

The proteomics highlights the structure, physiological function and interactions of the proteins in a cell or organism together with the alterations that attribute to the catalysis of varied biochemical pathways (Mishra 2011; Gupta et al. 2016). As a functional module, it is evident that several proteins play the role of enzymes in various biochemical reactions. They also act as antigens, antibodies, hormones and neurotransmitters (Mishra 2011; Sialana et al. 2018; He et al. 2018). Different techniques including protein separation, sequencing and the output correspond to the beginning of the proteomics.

The sample preparation in proteomic experiments needs to be highly accurate and well defined. There is no standard technique for sample preparation. The standardized protocol is influenced by the number of proteins in the sample, its complexity and the objectives. The sample extraction is a most vital step and involves extraction using solvents and detergents by tissue disruption technique and lyophilization (Macklin et al. 2020).

The crude protein is separated and isolated using gel-based and/or chromatographic techniques. PAGE, 1-DE and 2-DE are widely used for separating proteins. The 1-DE is employed to isolate proteins with the molecular weight ranging between 10 and 300 kDa. The SDS is used as detergent that denatures the secondary and

non-disulfide tertiary linkages, and attributes charge proportional to the volume binding with negative charges. This permits sieving effect based on the molecular weights (Chen et al. 2015). SDS-PAGE is commonly used to purify protein samples.

Principally, all proteins are separated by isoelectric pH (pI) and molecular size. The immobilized pH gradient (IPG) strips are employed for precise and stable pH gradient for the first-dimension IEF, followed by staining of gels and analysis of the protein spots by software. The individual spot is a polypeptide with intensity representing the quantity. The spot of choice can be excised using in-gel digestion with trypsin and identified using mass spectrometry (MS) (Gombar et al. 2017; Zhang et al. 2020). The diverse quantitative approaches include gel-based and non-gel-based approaches (Fig. 5.2). In the former, 2-D gel electrophoresis is replaced by ultra-modern 2-D Fluorescence Difference Gel Electrophoresis (DIGE). The cyanine dyes, Cy3 and Cy5, are used for differential labeling of similar proteins extracted from different samples. DIGE allows the analysis of multiple samples simultaneously, which is otherwise impossible with other techniques (Pineiro et al. 1999; Forné et al. 2009; Gebriel et al. 2014). In addition, the noticeable benefit of 2D-DIGE is to lessen the inter gel variability aiding in spot matching and identification in a snapshot, thus, precise quantification and capability to detect post-translational modifications (Alban et al. 2003). Recent studies implicated that 2-DE and MS have enabled the identification of protein mixtures. However, the detection of small samples and hydrophobic proteins is still facing problems using gel-based approach.

The 2-DE is more effective than the former, as it has high resolution and differentiates proteins based on isoelectric point, along with sieving effect based on molecular weights (Chen et al. 2015). 2-DE is used for protein expression profiling and cell map proteomics. The limitation of 2-DE is that it cannot detect low-molecular-weight proteins, and separation is by isoelectric point and size (Moseley

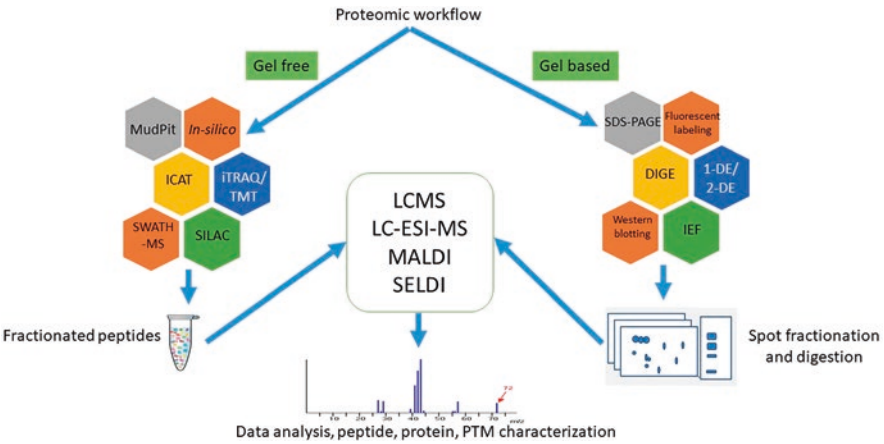

Fig. 5.2 A wide array of proteomic techniques used to understand proteomics signature in different organisms including fish

2001). The chromatographic techniques based on affinity, size exclusion, and ion exchange are also utilized to purify proteins. The western blotting and enzyme-linked immunosorbent assay are also used to recognize proteins. The ion-exchange chromatography utilizes the charge for the purification process, while size exclusion is based on the size or hydrodynamic volume. The two fundamental versions of size exclusion chromatography are gel permeation chromatography (uses organic solvents) and gel filtration chromatography (uses aqueous solvents) (Lecchi et al. 2003; Jungbauer and Hahn 2009). Affinity chromatography is yet another chromatographic technique used for protein separation, which is based on the interface with the immobilized ligand. In both 2-DE and non-2-DE, this technique plays a significant role by reducing the protein complexity (Marouga et al. 2005). The liquid chromatography (LC) is another powerful technique for protein separation and can analyze large and delicate biomolecules (Chen et al. 2007). The LC has been used to discover the novel therapeutic components and bioactive metabolites and its application in different therapies (Lai et al. 2001).

The proteins, once separated, are identified using mass spectrometry (MS), Edman sequencing and protein microarray (Pastwa et al. 2007), and this is a critical step in proteomics (Fig. 5.3). The MS is the finest analytical tool assisting in the identification of protein sequence (Martin and Nelson 2001). It also detects molecular weight, where the proteins are ionized and mass-to-charge ratios are calculated. The MS contains an analyzer, an ion source and a detector. The ESI and MALDI are used for ionization (Krishnankutty et al. 2016). In ESI, the power is activated in the protein sample to generate charged droplets that enhance the gaseous ion production to be determined using MS (Lahm and Langen 2000). The results are reproducible. Further, many categories could be combined to this. ESI can also be fixed to time-of-flight (TOF)-MS, quadruple, ion traps and Fourier transform ion cyclotron

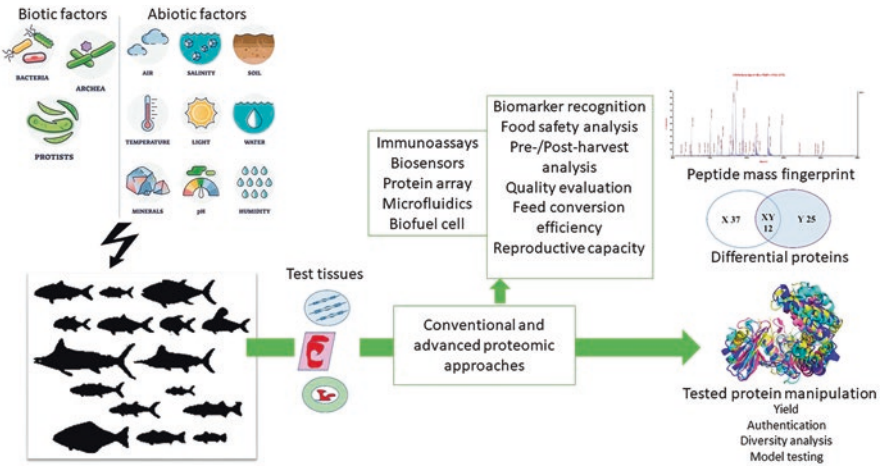

Fig. 5.3 A typical experimental setup in conventional and modern fish proteomics and its applications

resonance. Nevertheless, molecular imaging is not possible using ESI and needs large sample size. The multiple peaks attained due to several charged ions may interfere with the interpretation of the results (Chiou and Wu 2011). In MALDI, the chemical matrix is combined with peptides and spotted onto a metal multiwall microliter plate for a crystal lattice. The matrix chemicals pass the energy to the samples after absorbing it. Then, the peptide ions are sensed by a mass analyzer. MALDI produces mostly singly charged ions that help to determine the m/z values (Smith et al. 2006).

Recently, the emerged non-gel-based approaches are labeling techniques comprising isotope-coded affinity tags (ICAT), stable isotope labeling (SILAC), isobaric tagging (iTRAQ) and label-free techniques, namely, spectral counting and absolute quantitation (Hogstrand et al. 2002; Booy et al. 2005; Martyniuk and Denslow 2009; Ma et al. 2018) (Fig. 5.2). The two most prevalent ionization approaches are the matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI). For MALDI, the requisite is that samples must be in a crystalline state, while ESI directly ionizes the molecules in the solution, thus, pairing chromatographic separations with MS (Dietrich et al. 2015; Rossel et al. 2021). The mass analyzer distinguishes the ions based on its mass: charge ratio (m/z). These are directed to the detector by electric or magnetic fields. The mass analyzers popular in proteomics are time-of-flight (TOF), ion trap, quadrupole, Fourier transform ion cyclotron resonance (FT-ICR) and hybrid analyzers (TOF-TOF or Q-TOF) (Tay et al. 2006; Wang et al. 2007). In general, proteomics study includes fractionation of complex protein mixture and MS to attain protein sequence data and algorithms to examine and assemble these. Quantitative proteomics include mapping and spotting the differences in protein expression levels in normal and treated/diseased cells, at different time points or in different physiological state and so on.

The proteomic data, particularly generated through mass spectrometry analysis, are well established and growing rapidly. The data generated are stored for later use. The data generated include huge volumes of raw experimental data and inferred biological results, which have broad applications and are significant for clinicians (improve medical diagnostics and treatment tools), biologists (elucidate the mode of action of specific proteins) and researchers (new software tools for the evaluation and elucidation of MS data). The public *databases*, *data sets* and *data repositories*, at highest level, exist to make data accessible to all the users. Nevertheless, it is imperative to understand that proteomics data are diverse and used by different users for different requirements and applications. No data repository will provide all data to all users and may not be possible to evaluate with same set of criteria (Riffle and Eng 2009). Generally, the tandem mass spectrum explored against a reference protein database and the candidate peptides within the given mass tolerance are recorded. The highest scoring peptide is considered as the identification result and is stated as peptide-spectrum match (PSM). The identified peptides are filtered within a given false discovery rate (FDR) threshold and used to acquire the final protein report. The FDR control is accomplished through the target-decoy approach (TDA), in which MS/MS data are searched against the database formed by the combination of target and decoy proteins, and the FDR is estimated accordingly (Wang et al. 2023).

Proteomics allows in understanding complex diseases, recognition of biomarkers and discovery of new proteins as drugs (Damodaran et al. 2006; Mishra 2011). With developments in gene expression analysis, there is a wider application of novel proteomic techniques in fisheries including fish endocrinology studies.

The rich proteomic data support data-driven hypothesis generation, biological knowledge discovery, new scientific insights and so on, and to facilitate these, several protein-related bioinformatics databases, query facilities and data analysis software tools were developed, which establish and offer biological annotations for proteins to maintain sequence, structural, functional and evolutionary analyses in the context of pathway, network and systems biology (http://www.oxfordjournals.org/our_journals/nar/database/cap/). With the advent of genomic sciences and Next-Generation Sequencing (NGS) technologies, new protein bioinformatics databases are also gaining momentum, and the challenge faced by computational biologists to support the *Big Data* is striking (Metzker 2010). Selecting an appropriate reference database is critical. This improves precision, recall of identification results and reduced time. The reference database must comprise as many proteins that exist in the given sample with minimum or no irrelevant data (Wang et al. 2023). The limitations commonly observed include constructing an accurate database before the database search. For instance, in the metaproteomics that concentrates on identifying proteins in a complex biological sample (microbial populations in a specific environment and so on), it is tough to build a precise proteome database containing exact sample- or species-specific proteins for each experiment. Similarly, only a small proportion of proteins in the databases may be of real significance in the downstream studies, while too many irrelevant proteins are inevitably included in the database. And this in turn will hamper the database search performance.

5.2 Data Sets

The exhaustive data produced as a result of mass spectrometry or shotgun proteomics comprise mass-to-charge ratios of peptides and fragments. These databases are thoroughly searched using different search engines. The search engines lead to the peptide matches from in silico digested proteins to those with MS. A number of software applications are currently available for database searching. These include SEQUEST, MaxQuant/Andromeda, Sequent, Mascot, Comet and Tandem (Adkins et al. 2002; Paulo 2013). Nevertheless, most search devices do not produce matching data as these operate on differentiation algorithms and recording functions, creation integration and data comparison from numerous studies and experiments. As a result, the identification of peptides by data search is time consuming (Carr et al. 2004). High-quality data make the data search effective and save time. Furthermore, using accurate mass to measure ion fragments shortens database explorations and produces accurate results (Nesvizhskii et al. 2003).

5.2.1 Bioinformatics and Computational Tools

Bioinformatics pools the methods used in the collection, storage, identification, analyses and correlation of composite data. The goal being to offer the precise means to explain normal biological processes, dysfunctions leading to diseases and approaches to discover cure and overcome the hurdles.

Bioinformatics analyses use novel proteomics algorithms to achieve the absolute results (Vihinen 2001). Regulating this massive quantity of data and discovering the association between other omics technologies (metabolomics and genomics) remains a challenging task. The evaluation of proteomics data is thought-provoking because of the factors used in processing and quality valuation. The critical challenge is how to analyze massive data and create real biological understanding (Domon and Aebersold 2006). The proteins identified are validated using several computational tools. Several databases, data sets and repositories have been identified (Table 5.1).

Table 5.1 An outline of protein bioinformatics databases (Chen et al. 2017)

Category	Database	Website
Sequence databases	The consensus CDS protein set database	https://www.ncbi.nlm.nih.gov/CCDS/CcdsBrowse.cgi
	DNA Data Bank of Japan	http://www.ddbj.nig.ac.jp/
	European nucleotide archive	http://www.ebi.ac.uk/ena
	GenBank nucleotide sequence database	https://www.ncbi.nlm.nih.gov/genbank/
	NCBI reference sequence database	https://www.ncbi.nlm.nih.gov/refseq/
	Database of computationally identified transcripts from the same locus	https://www.ncbi.nlm.nih.gov/unigene/
	Universal protein resource	http://www.uniprot.org/
2D gel databases	2-DE database at Universidad Complutense de Madrid, Spain	http://compluyeast2dpage.dacya.ucm.es/
	2-DE database at Nanjing Medical University, China	http://reprod.njmu.edu.cn/cgi-bin/2d/2d.cgi
	2-DE database at Swiss Institute of Bioinformatics, Switzerland	http://world-2dpage.expasy.org/swiss-2dpage/
	The world-2DPAGE database	http://world-2dpage.expasy.org/repository/
3D structure databases	Database of protein disorder	http://www.disprot.org/
	Database of intrinsically disordered and mobile proteins	http://mobidb.bio.unipd.it/
	Database of comparative protein structure models	http://modbase.compbio.ucsf.edu/modbase-cgi/index.cgi
	Protein data bank at Europe	http://www.ebi.ac.uk/pdbe/
	Protein data bank at Japan	http://pdbj.org/
	Pictorial database of 3D structures in the Protein Data Bank	http://www.ebi.ac.uk/pdbsum/
	Protein model portal of the PSI-nature structural biology knowledgebase	http://www.proteinmodelportal.org/
	Protein Data Bank at RCSB	http://www.pdb.org/
	Database of annotated 3D protein structure models	http://swissmodel.expasy.org/repository/
Chemistry databases	The binding database	http://www.bindingdb.org/
	Database of bioactive drug-like small molecules	https://www.ebi.ac.uk/chembldb
	Drug and drug target database	http://www.drugbank.ca/

(continued)

Table 5.1 (continued)

Category	Database	Website
Enzyme and pathway databases	MetaCyc database of metabolic pathways BioCyc collection of pathway/genome databases	http://www.biocyc.org/
	Braunschweig enzyme database	http://www.brenda-enzymes.org
	Enzyme nomenclature database	http://enzyme.expasy.org/
	A knowledgebase of biological pathways and processes	http://www.reactome.org/
	SABIO-RK: biochemical reaction kinetics database	http://sabiork.h-its.org/
	A signaling pathway resource with multi-layered regulatory networks	http://signalink.org/
	UniPathway: a resource for the exploration of metabolic pathways	http://www.unipathway.org
Family and domain databases	Structural and functional annotation of protein families	http://gene3d.biochem.ucl.ac.uk/Gene3D/
	High-quality automated and manual annotation of proteins	http://hamap.expasy.org/
	Integrated resource of protein families, domains and functional sites	http://www.ebi.ac.uk/interpro/
	The PANTHER classification system	http://www.pantherdb.org/
	The Pfam protein families database	http://pfam.xfam.org/
	A whole-protein classification database	http://pir.georgetown.edu/pirwww/dbinfo/pirsf.shtml
	Protein motif fingerprint database	http://www.bioinf.manchester.ac.uk/dbbrowser/PRINTS/
	Protein domain families database	http://prodom.prabi.fr/prodom/current/html/home.php
	Database of protein domains, families and functional sites	http://prosite.expasy.org/
	Automatic hierarchical classification of proteins	http://www.protonet.cs.huji.ac.il/
	Simple modular architecture research tool	http://smart.embl.de/
	Superfamily database of structural and functional annotation	http://supfam.org
	TIGRFAMs protein family database	http://www.jcvi.org/cgi-bin/tigrfams/index.cgi
Gene expression databases	Database for gene expression evolution	http://bgee.unil.ch
	Database of gene expression profiles	http://cleanex.vital-it.ch/
	Search portal to normalized and curated expression data from Genevestigator	http://genevisible.com/search
	Database of differential and baseline expression	http://www.ebi.ac.uk/gxa/home
Genome annotation databases	Ensembl eukaryotic genome annotation database	http://www.ensembl.org/
	Ensembl bacteria genome annotation database	http://bacteria.ensembl.org/
	Ensembl fungi genome annotation database	http://fungi.ensembl.org/
	Ensembl metazoa genome annotation database	http://metazoa.ensembl.org/
	Ensembl plants genome annotation database	http://plants.ensembl.org/
	Ensembl protists genome annotation database	http://protists.ensembl.org/
	Database of genes of genomes in the reference sequence collection	https://www.ncbi.nlm.nih.gov/gene
	Kyoto encyclopedia of genes and genomes	http://www.genome.jp/kegg/
	Bacterial Bioinformatics Resource Center	http://patricbrc.org/
	UCSC genome bioinformatics	http://genome.ucsc.edu
	Bioinformatics resource for invertebrate vectors of human pathogens	http://www.vectorbase.org/
	WormBase ParaSite	http://parasite.wormbase.org

(continued)

Table 5.1 (continued)

Category	Database	Website
Organism-specific databases	ArachnoServer: spider toxin database	http://www.arachnoserver.org
	Candida genome database	http://www.candidagenome.org/
	ConoServer: cone snail toxin database	http://www.conoserver.org/
	Comparative toxicogenomics database	http://ctdbase.org/
	Central resource for dictyostelid genomics	http://dictybase.org/
	EchoBASE—an integrated post-genomic database for *E. coli*	http://www.york.ac.uk/res/thomas/
	Escherichia coli strain K12 genome database	http://www.ecogene.org/
	The European Hepatitis C Virus database	https://euhcvdb.ibcp.fr/euHCVdb/
	Eukaryotic pathogen database resources	http://eupathdb.org/eupathdb/
	A database of drosophila genes and genomes	http://flybase.org/
	A database on genes, functions and related diseases	http://genatlas.medecine.univ-paris5.fr/
	The human gene database	http://www.genecards.org/
	Integrated environment for the analysis of microbial genomes	http://genodb.pasteur.fr/cgi-bin/WebObjects/GenoList
	A comparative resource for plants	http://www.gramene.org/
	H-Invitational database	http://www.h-invitational.jp/
	HUGO gene nomenclature committee database	http://www.genenames.org/
	The human protein atlas	http://www.proteinatlas.org/
	A database of human unidentified gene-encoded large proteins	http://www.kazusa.or.jp/huge/
	Legionella pneumophila genome database	http://genolist.pasteur.fr/LegioList/
	Mycobacterium leprae genome database	http://mycobrowser.epfl.ch/leprosy.html
	Maize genetics and genomics database	http://www.maizegdb.org/
	Mouse genome database	http://www.informatics.jax.org/
	MICrobial advanced database organization	http://genome.jouy.inra.fr/cgi-bin/micado/index.cgi
	Online mendelian inheritance in man	http://www.omim.org/
	Exploring the universe of human proteins	http://www.nextprot.org/
	The portal for rare diseases and orphan drugs	http://www.orpha.net/consor/cgi-bin/home.php?Lng=GB
	The pharmacogenomics knowledgebase	http://www.pharmgkb.org
	The scientific resource for fission yeast	http://www.pombase.org/
	The pseudomonas genome database	http://www.pseudomonas.com/
	Rat genome database	http://rgd.mcw.edu/
	A database of rodent unidentified gene-encoded large proteins	http://www.kazusa.or.jp/rouge/
	Saccharomyces genome database	http://www.yeastgenome.org/
	The arabidopsis information resource	http://www.arabidopsis.org/
	Mycobacterium tuberculosis strain H37Rv genome database	http://tuberculist.epfl.ch
	C. elegans and related nematodes genetics and genomics database	http://www.wormbase.org/
	Xenopus laevis and *tropicalis* biology and genomics resource	http://www.xenbase.org/
	The Zebrafish model organism database	http://zfin.org/

Table 5.1 (continued)

Category	Database	Website
Phylogenomic databases	Database of orthologous groups and functional annotation	http://eggnog.embl.de/
	Database of homologous genes from fully sequenced organisms	http://pbil.univ-lyon1.fr/databases/hogenom/home.php
	Homologous vertebrate genes database	http://pbil.univ-lyon1.fr/databases/hovergen.html
	Eukaryotic ortholog groups with inparalogs	http://inparanoid.sbc.su.se/
	Kyoto encyclopedia of genes and genomes orthology	http://www.genome.jp/kegg/
	The OMA orthology database	http://omabrowser.org/
	Database of orthologous groups	http://cegg.unige.ch/orthodb6
	Database for complete catalogs of gene phylogenies (phylomes)	http://phylomedb.org/
	Database of animal gene trees	http://www.treefam.org
Polymorphism and mutation databases	Single-nucleotide variation and disease association database	https://hive.biochemistry.gwu.edu/tools/biomuta/
	Database of short genetic variations	https://www.ncbi.nlm.nih.gov/SNP/
	Domain mapping of disease mutations	http://bioinf.umbc.edu/dmdm/
Protein–protein interaction databases	The biological general repository for interaction datasets	http://thebiogrid.org
	Database of interacting proteins	http://dip.doe-mbi.ucla.edu/
	IntAct molecular interaction database	http://www.ebi.ac.uk/intact/
	The molecular INTeraction database	http://mint.bio.uniroma2.it/mint/
	Search tool for the retrieval of interacting genes/proteins	http://string-db.org
Proteomic databases	The MaxQuant DataBase	http://maxqb.biochem.mpg.de/mxdb/
	Protein abundance across organisms	http://pax-db.org
	PeptideAtlas	http://www.peptideatlas.org
	PRoteomics IDEntifications database	http://www.ebi.ac.uk/pride
	Protein mass spectra EXtraction	http://promex.pph.univie.ac.at/promex/
PTM databases	The human DEPhOsphorylation database	http://www.koehnlab.de/depod/index.php
	Protein post-translational modifications (PTMs) in systems biology context	http://research.bioinformatics.udel.edu/iptmnet/
	The arabidopsis protein phosphorylation site database	http://phosphat.uni-hohenheim.de
	Database of S/T/Y phosphorylation sites	http://phospho.elm.eu.org
	Database of experimentally verified in vivo protein phosphorylation sites	http://www.phosphogrid.org
	Phosphorylation site database	http://www.phosphosite.org
	Database of glycomics and glycobiology	http://www.unicarbkb.org/
Ontology	Gene ontology	http://www.geneontology.org/
	Protein ontology	http://pir.georgetown.edu/pro/pro.shtml

(continued)

Table 5.1 (continued)

Category	Database	Website
Specialized protein databases	Allergome: platform for allergen knowledge	http://www.allergome.org/
	Carbohydrate-active enZYmes database	http://www.cazy.org/
	ESTerases and alpha/beta-hydrolase enzymes and relatives database	http://bioweb.ensam.inra.fr/ESTHER/general?what=index
	Information system for G protein-coupled receptors (GPCRs)	http://www.gpcr.org/7tm/
	The international ImMunoGeneTics information system	http://www.imgt.org/
	MEROPS protease database	http://merops.sanger.ac.uk/
	Moonlighting protein database	http://www.moonlightingproteins.org/
	Characterized lignocellulose-active proteins of fungal origin	https://mycoclap.fungalgenomics.ca/mycoCLAP/
	The peroxidases database	http://peroxibase.toulouse.inra.fr/
	The restriction enzyme database	http://rebase.neb.com/rebase/rebase.html
	Transporter classification database	http://www.tcdb.org/
Other [miscellaneous] databases	Database of chimeric transcripts and RNA-seq data	http://chitars.bioinfo.cnio.es/
	Database of relative evolutionary importance of amino acids within a protein sequence	http://mammoth.bcm.tmc.edu/ETserver.html
	Wiki portal for the annotation of gene and protein function	http://en.wikipedia.org/wiki/Portal:Gene_Wiki
	Database of phenotypes from RNA interference screens in Drosophila and *Homo sapiens*	http://genomernai.dkfz.de/GenomeRNAi/
	Proteolytic event database	http://www.proteolysis.org/
	The Stanford online universal resource for clones and ESTs	http://smd.princeton.edu/cgi-bin/source/sourceSearch

These databases are not the exhaustive list. There are other databases too. Protein pathways are the assembly of internal cell reactions with definite biological effects. For protein pathways, diverse tools and databases are accessible (Aslam et al. 2016). The Kyoto Encyclopedia of Genes and Genomes, Pathway Knowledge Base Reactome, BioCarta and Ingenuity pathway databases are other examples that have extensive data on metabolism, signaling and interactions (Croft et al. 2010; Kanehisa et al. 2012). Exceptional databases for signal transduction pathways (GenMAPP) or protein analysis through evolutionary interactions (PANTHER) are also available (Mi et al. 2007; Schaefer et al. 2009). Additionally, databases such as Netpath, for cancer-related pathways, are available to detect specific proteins in specific cancer types (Kandasamy et al. 2010). Particulars about protein interactions in complex systems can be found in MINT, BioGRID, HRPD and IntAct (Chatr-Aryamontri et al. 2007; Kerrien et al. 2012; Schmidt et al. 2014). The STRING database links other databases and aids in literature mining. This also helps in understanding protein networks drawn based on the genes and their interactions (Glaab et al. 2012; Franceschini et al. 2012).

As suggested earlier, it is difficult to search and narrow down the database search to fish proteins specifically. A few studies have been reported in fish proteins. Fish collagen has a wide spectrum application as food additive and nutricosmetics due to its high water-holding capacity and antioxidant activity (Rodrigues et al. 2018). It

also exhibits gelling and antimicrobial properties (Li et al. 2019). The peptides obtained from hydrolyzed collagen were reported to have anti-inflammatory properties (Wang et al. 2018), and the peptides may find applications as anti-arthritic and antifreezing/cryoprotective agents in food sector and biomedicine (Wu et al. 2018; König et al. 2018). Nuñez et al. (2020) used bioinformatics tool to characterize the collagen from bovine, porcine, chicken, trout and salmon, in terms of its domain and physicochemical properties calculated in silico, based on the reported sequences in Uniprot databases. The authors also performed in silico hydrolysis of peptide sequence using subtilisin (Alcalase) and characterized and compared these reported peptide sequences with varying biological activities. The study initially included search for collagen type 1 from these sources using UniProt databases for α-1 and α-2 chains. Annotations defining domains and other features are presented for certain sequences. The researchers use these according to the similarity and based on multiple alignment made using Geneious 9.1.8 package (http://www.geneious.com). The limitations observed were the limited resources (α-3 in salmon and trout were not available), and Saito et al. (2001) reported only one fragment for trout (NCBI accession BAA 33381). Similarly, the hydrolysis simulation and end product as collagen peptides were defined according to BIOPEP (C-terminus Try, Phe, Leu, Trp and Ser, and N-terminus Val) (http://www.uwm.edu.pl/biochemia/index.php/en/biopep). Redundant and shorter sequences were eliminated from the study. The results indicated that bovine, porcine fish (trout and salmon) collagen can be used interchangeably due to their high identity. The results were identical and consistent with the evolution of proteins in related species.

Another aspect of the application of bioinformatics in fisheries and aquaculture is to improve nutritional value and livelihood security (Fig. 5.4). Genomic research

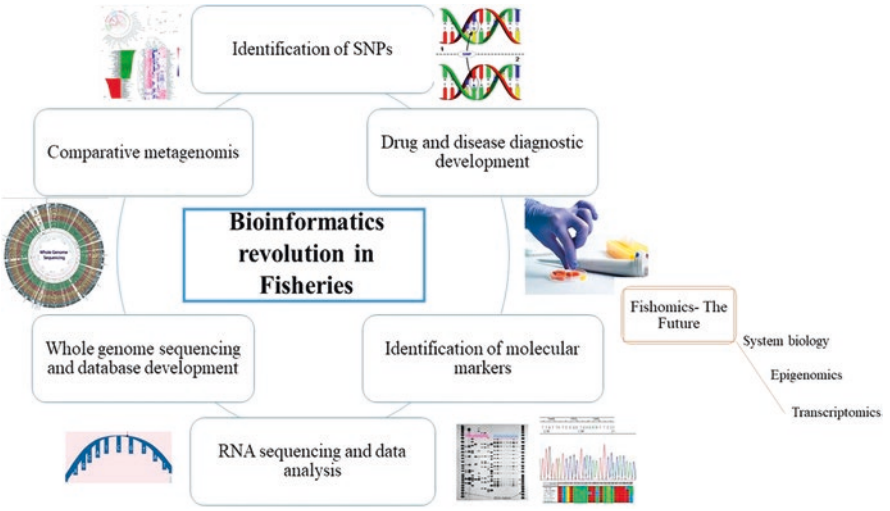

Fig. 5.4 Various applications of bioinformatics in aquaculture

has added significantly toward the growth of beneficial technologies in aquaculture together with Next-Gen technologies for generating oceanic data.

Techniques such as DIGE and ICAT are effectively used for the analysis of proteomic response to hypoxia in Japanese rice fish and various diseases in Atlantic salmon (Noor et al. 2021; Booy et al. 2005). Fish proteomics is also beneficial in understanding the response to reproductive steroids and environmental mimics. Fish physiology and toxicology need to be integrated with genomics, proteomics and metabolomics for an in-depth understanding (Gebriel et al. 2014; Kolder et al. 2016; Schmitz et al. 2017; Li et al. 2022) (Table 5.2). Several studies have reported the relative fish proteomics (Forné et al. 2010), proteomics and fish immunity (Ye et al. 2018), proteomics and fish quality (Rodrigues et al. 2018), fish proteomics and food security (Nissa et al. 2021a), fish proteomics in farm condition assessment (Carrera et al. 2020), semen proteomics (Ciereszko et al. 2017), fish proteomics for food authentication (Ortea et al. 2020) and proteomics and fish diagnostics (Moreira et al. 2021). Therefore, these *-omics* give a holistic approach to visualize the interplay of molecules and bridge the gap between genotype and phenotype (Canzler et al. 2020).

Aquaculture is a multi-billion industry that has gained immense attention all over the world not only for its contribution to economic basket but also as a cheap source of protein; hence, the economic loss due to disease outbreak in this sector is a major concern. Therefore, immune response to biotic invasions and drug research and development is highly significant (Saleh et al. 2019). However, it is a time-consuming complex processing and requires huge capital investment (Assefa and Abunna 2018). It initiates with target identification (genes, proteins and RNA) that might bind with lead compounds. Several tools, software and databases are used for this purpose in aquaculture. These include molecular docking tools (AUTODOCK 4.2/AUTODOCKVINA, UCSF DOCK (DOCK 6 AND DOCK 3), GOLD, FLEXX, FRED, GLIDE, PATCHDOCK, ICM-, IGEMDOCK, MOLEGRO VIRTUAL DOCKER (MVD), HIGH AMBIGUITY DRIVEN BIOMOLECULAR DOCKING (HADDOCK), SWISSDOCK); visualization tools (PYMOL, CHIMERA, DEEPVIEW SWISS-PDBVIEWER, VISUAL MOLECULAR DYNAMICS, RASMOL); binding site prediction tools (LIGSITE^{CSC}, FINDSITE$^{COMB2.0}$, 3DLINGANSITE, ACTIVE SITE PRODUCTION, CASTP, SITEMAP); target identification and validation software (BIOEDIT—BIOLOGICAL EDITOR), CLUSTAL OMEGA, BLAST (BASIC LOCAL ALIGNMENT SEARCH TOOL), EMBOSS (EUROPEAN MOLECULAR BIOLOGY OPEN SOFTWARE SUITE), SMART (SIMPLE MODULAR ARCHITECTURE RESEARCH TOOL); protein modeling tools (MODELLER, SWISS-MODEL, PRIME, PHYRE2, I-TASSER); commercial software packages for drug design (DISCOVERY STUDIO, OPENEYE, ICM); molecular dynamics software (GROMACS, CHARMM, NAMD, AMBER, DESMOND) and drug and chemical databases (PUBCHEM, CHEMBLDB, DRUGBANK, ZINC, GVBIO DATABASES, CHEMSPIDER, BINDINGDB, THERAPEUTIC TARGET DATABASE) (Noor et al. 2021). The targeted therapy requires validation of biomolecules at molecular, cellular and animal level. The lead candidates thus identified are optimized for optimum potency, effectiveness,

Table 5.2 Proteomics in fish health state with respect to food quality, biomarkers, species identification (Jaiswal et al. 2023)

Technique deployed	Aim of study	Model/fish	Tissue/cell type
SDS-PAGE, 2-DE	Cryopreservation-induced protein phosphorylation	*Cyprinus carpio*	Sperm
2-DE MALDI-TOF	Screen the effect upon cryopreservation	*Centropristis striata*	Semen, sperm
2-DE, ESI- MS/MS	Analysis of cytosolic biomarker	*Danio rerio*	Hepatocyte
SDS-PAGE, 2-DE	Identification of freshness indicator	*Dicentrarchus labrax*	Muscle
MALDI-TOF, MS	Identification of shrimp species	Various spp.	Whole specimen
2-DE, MALDI-TOF	Postmortem changes evaluation	*Sparus aurata*	Muscle
2-DE, MALDI-TOF	Development of species-specific proteomic marker	*Sperata seenghala* *Sperata aor*	Muscle sarcoplasm
MALDI-TOF MS	Discrimination of freshwater fish species	*Alosa agone Scopoli* *Coregonus macrophthalmus Nüsslin* *Rutilus rutilus*	Muscle and liver tissues
2-DE and MALDI-TOF MS	Analysis of parvalbumin fractions to determine species identification	Hake and grenadier species	Muscle extract
NanoESI-IT tandem (MS/MS)	Identification of species-specific peptides	*Mytilus edulis* *Mytilus galloprovincialis* *Mytilus trossulus*	Whole specimen
2-DE-IEF, MALDI-TOF-MS	Identification and characterization species-specific polypeptides	*Merluccius merluccius* *M. australis* *M. hubbsi* *M. gayi* *M. capensis*	Sarcoplasm
UPLC-MS/MS with SWATH	Proteomics for species authentication	Alaska Pollock Atlantic cod Greenland halibut	Whole specimen
MudPIT	Investigate proteomics-based dimorphism	*Danio rerio*	Gonads
Quantitative proteomics	Develop library for ecological proteomics	*Gasterosteus aculeatus*	Gills
2-DE coupled with imaging	Monitor intra-species contamination	Rainbow trout and Others	Cell lines
LC-MS	Accesses comparative proteomic of selective breeding	*Oncorhynchus kisutch*	Skeletal muscle
MS	Identification of species	*Thunnus* species	Muscle
2D-DIGE, ESI-MS	Protein abundance in extracellular medium	*Cyprinus carpio*	Sperm

(continued)

Table 5.2 (continued)

Technique deployed	Aim of study	Model/fish	Tissue/cell type
SWATH/ DIA-MS	Monitor species-specific proteome level	*Acanthochromis polyacanthus*	Liver and brain
MALDI-TOF MS	Species-specific identification of fish eggs	Diverse specimen	Eggs
LC-MS/MS	Establishment of a non-invasive method for welfare indicators	*Sparus aurata*	Skin mucus
Nano-LC-MS/ MS	Investigate protein diversity in skin mucus and venom	*Scorpaena plumieri*	Skin mucus and venom
pH gradient directed 2-DE	Proteome-based species identification	*Tetraodontidae* spp.	Muscle
1D and 2D, LC–MS/MS	Investigate fish venom composition	*Neotrygon kuhlii*	Barb tissue
MS	Biomarker identification for inflammation	*Salmo salar*	Plasma
iTRAQ, LC–MS/ MS	Evaluate the effects of super-chilling on storage and shelf-life	*Coregonus peled*	Muscle
Label-free proteomics	Reveal freshness-related proteins	*Lateolabrax japonicus*	Fillets
LFQP, MS/MS	Investigate quantitative molecular phenotyping	*Oreochromis mossambicus*	Gills
2D SDS-PAGE	Showcase environmental contamination assessments	*Oreochromis niloticus*	Bile
2D-DIGE with MS	Population proteomics	*Merluccius merluccius*	Liver and brain
2D-DIGE and MS	Postmortem storage	*Dicentrarchus labrax*	Muscle
2-DE, MALDI-TOF/TOF	Proteins related to freezability	*Carassius auratus)*	Seminal plasma
Liquid fractionation-coupled MS	Identification of the potential bioactive proteins	*Channa striata*	Mucus
2D-DIGE, LC-MS/MS	Screen the proteomics upon chemotherapeutics	*Pangasianodon hypophthalmus*	Muscle and blood
MALDI-MS	Differentiation and authentication	Diverse fish species	Skin
iTRAQ	Phosphoproteomic-based quantitative analysis	*Ctenopharyngodon idella*	Muscle
PAGE, MALDI-TOF	Protein degradation upon storage	*Mylopharyngodon piceus*	Muscle
LC-MS/MS	Proteomic markers upon stunning stress and stress-induced textural tenderization	*Hypophthalmichthys molitrix*	Fillets

selectivity, pharmacokinetics and pharmacodynamics. Pre-clinical and clinical trials are conducted for validation before it is marketed. The majority of drug tests are tried on fish models such as zebra fish (*Danio rerio*), Medaka (*Oryzian latipes* or Japanese rice fish), Takifugu (*Takifugu rubripes* or pufferfish), three-spined stickleback (*Gasterosteus aculeatus*), mouse (*Mus musculus*) and so on. These are followed by clinical trials in test ponds. Briefly, the bioinformatics or computer-aided techniques used in drug design and discovery are:

- Structure-based virtual screening
- Molecular docking
- Pharmacophore modeling
- Ligand-based virtual screening
- Homology modeling
- Molecular dynamics
- 3D quantitative structure–activity relationship
- ADMET (Adsorption, Distribution, Metabolism, Excretion and Toxicity)

Along with drug designing, the lead molecules are tried as growth stimulators, color enhancers, etc. Bioinformatics could propose enormously toward next-generation research and development in aquaculture, thus contributing to a future smart aquaculture. Apart from these, proteomics is also used in fish biology research to comprehend developmental biology, physiology, disease/stress and species recognition. Nevertheless, this area of research is not as prominently developed in fisheries as in humans or other organisms. Initially, some model organisms such as zebrafish (*Danio rerio*), salmonids and cyprinids were used for proteomics research. Absence of genetic information for majority of fish species restricts the general application of proteomics in fishery research. It is challenging to study the potential applications of aquatic organisms (specifically, fish and shellfish), which are known to possess unique physiological characteristics (Forné et al. 2010; Nynca et al. 2014). A total of 45 different fish proteome analyses were published over 40 different fish species, with major ones being zebrafish, rainbow trout and Atlantic salmon (Forné et al. 2010). There is a vast void in the fish proteomics and several species have not been recorded; hence, there is a need for detailed fish proteomic analyses.

5.2.2 Advances and Applications of Bioinformatics Tools

Over the last 10 years, there has been a massive inflow of MS-based proteomics, and hence, scientific communities are compelled to improve and develop algorithms, tools and repository databases. Despite the challenges, the Human Genome Project and Human Proteome Organization have aided in the advancements of proteomics. Several standalone software and comprehensive databases have assisted in the formation of integrated omics pipeline and meta-analysis workflow that has added to the know-how of the disease pathobiology, biomarker discovery and new therapeutic modalities. For shotgun proteomics (Data-Dependent Acquisition), several

user-friendly software is established that can examine the pre-processed data to provide mechanistic insights of the disease/condition, while in Data-Independent Acquisition, spectral library is built to identify the therapeutic targets. Besides, in the age of big data analysis the implications of machine learning and cloud computing are adding robustness, rapidness and in-depth proteomics data analysis. It is also important to note that no repository could store both raw data and processed results at the same time. The uncertainty of where to submit the data set and in what form has also contributed to these advancements. Not only them, the conveniences and data-friendliness for consumers have also contributed toward it.

The ProteomeXchange consortium emerged as the lead actor to regulate data sharing and submission between proteomics resources. This was established in 2006 to provide globally coordinated standard data submission and dissemination pipelines involving the main proteomics repositories and to encourage open data policies in the field (Vizcaíno et al. 2014), thus overcoming the challenges. ProteomeXchange started to accept regular data submission from June 2012 and currently has over 685 ProteomeXchange data sets (>32 Tb of data) (Vizcaíno et al. 2014).

Among the existing MS proteomics repositories, PRIDE (Proteomics IDEntifications) databases (European Bioinformatics Institute, EBI, Cambridge, UK; http://www.ebi.ac.uk/pride) and PeptideAtlas (Institute for Systems Biology, ISB, Seattle, WA; http://www.peptideatlas.org) are two of the most prominent. These chiefly focus on tandem MS (MS/MS) data storage. PRIDE represents the information as originally investigated by the researcher (primary source) and PeptideAtlas are reprocessed data through a common pipeline (Trans-Proteomic Pipeline) and constitute secondary resource. The ISB set up the first repository for selected reaction monitoring (SRM) data, PASSEL (PeptideAtlas SRM Experiment Library, http://www.peptideatlas.org/passel/). Other advanced resources dedicated for storing MS proteomics data include Global Proteome Machine Database (GPMDB, data reprocessed using search enginer X!Tandem). At higher abstraction level, resources such as UniProt and neXtProt are integrating proteomics results from different sources. The stakeholders other than PRIDE and PASSEL (primary) and PeptideAtlas and UniProt (secondary) include bioinformaticians, investigators (HUPO Human Proteome Project) and representatives from proteomic data publishers. Therefore, the prime aim of ProteomeXchange (PX) is to offer a common framework and infrastructure for the consistent proteomic resources, provide user-friendly data deposition and exchange procedures (Vizcaíno et al. 2014).

Further, with the advancements in IT and bioinformatics, there is a considerable interest toward filling the lacunae about the molecular information on important commercial fish species. The whole-genome sequencing has improved the success rate of the global fisheries sector considerably in the last few decades. The first fish species subjected to whole-genome sequencing was the Japanese puffer fish (*Fugu rubriceps*) (Aparicio et al. 2002). Since the sequencing era took a start and as per the data till the year 2019, the whole-genome sequences of more than 500 fish species are available in the public domain (NCBI). According to the Genome 10K project initiated by a consortium of biologists and genome scientists, the goal is to

sequence around 4000 fish species to apprehend evolutionary process in vertebrates and protect the endangered species.

The advent of high-throughput proteomics helps in identifying proteomic footprints for species recognition, authentication and traceability to ensure food safety, biomarker discovery and welfare, pathology and disease diagnosis, stress management, reproductive fitness, feed efficiency and feed conversion, aquatic pollution and biomonitoring, depiction of novel potent biomolecules and so on (Jaiswal et al. 2023). Since this area of research is gaining interest and lots of studies are being undertaken, novel innovations are happening in this field. Seeing the developments, it may be hypothesized that more and more innovations with databases and secondary data are yet to happen. Artificial intelligence is yet one such innovation that has influenced all sectors of life, and -*omics* are no exceptions.

5.3 Artificial Intelligence and Protein Structure

Beyond -*omics* data, other modality data are also facing rapid growth in all sectors. With the available data growing larger and broader, an ensuing technical concern is how to integrate and synthesize them. The computational algorithms and tools are required to excise meaningful data, which is a conceptual departure from classical statistics. Artificial intelligence (AI) or machine learning (ML), specifically deep learning (DL) (a subset of machine learning), a substantially new perspective, is flexible and well suited to address heterogeneous and unstructured data. It has a high propensity to discover non-linear and high-dimensional relationships in multimodal data. Over a few years, the AI has shifted from theoretical research to real-world applications. ML algorithms learn from the data these are exposed to and make predictions without being obviously programmed for the specific applications. DL creates deep neural networks consisting of many layers and aid in achieving unprecedented accuracy for various AI tasks including image recognition. These networks go beyond ML by learning complex features and "embeddings" of the data. To explain, a classical ML algorithm learn to distinguish flowers based on measured characteristics such as the length and the width of the sepals and petals, DL would deduce such features itself when exposed to images of different flower classes (Mann et al. 2021). General ML applications to proteomics began in 2000, while DL entered in 2017 and since then it has taken the major floor in proteomics workflow and interpretability. DL-based prediction tools such as deep neural networks (DNNs), recurrent neutral networks (RNNs), convolutional neural networks (CNNs), variational autoencoders (VAEs) and associated tools have proven to be highly appropriate and reliable in generating results (Liu et al. 2020). Crowdsourcing (NCI-CPTAC DREAM) is yet another tool that could be used for this purpose (Yang et al. 2020). Authors also emphasized that AI is essential to measure the proteome than transcriptome or other proxies. The recent DL breakthroughs in text mining are also a noteworthy application. Holistic understanding of biology necessitates an integrative approach of all data types (-*omics*, imaging, chemical, healthcare, etc.) and uncovers their complex interrelationship. ML, DL and NLP also assist in

biomedical data mining and generating biological and clinical insights including identifying biomarkers in the form of graphs (Clinical Knowledge Graph, CKG).

The field of artificial intelligence-assisted protein design is not a novel area. It has been used by academic researchers like David Baker and his colleagues at Institute for Protein Design at University of Washington. Together with his team Cyrus, Monod and Arzeda, they have developed several powerful tools for protein design powered by ML algorithms (deep learning). Artificial intelligence (AI) has solved a biggest challenge such as predicting the curling of proteins from a linear chain of amino acids to 3D shapes that allow them to carry out life's task. This has become the primary advancements in the series to come. Application of machine learning (ML) in engineering novel functional proteins achieved a great momentum in the recent past. This is just another latest change in the steady flurry of researches (Profluent received seed funding of US $9 million on 26 January 2023). Generate Biomedicine signed a US $50 million drug development deal with Amgen; Arzeda drew US $33 million in series B funding to support its ongoing protein design programs; computational company Cradle received US $5.5 million as seed investment and Monod Bio launched with US $25 million in August 2023, all these prove the importance of ML and artificial intelligence (AI)-based computational tools is gaining in predicting real-world protein structures. AlphaFold 2, an algorithm developed by scientists at DeepMind, predicts the protein structure on the basis of amino acid sequence (launched in July 2021). Presently, it is used by structural biologist as a routine tool and over 200 million structures have been predicted using this tool in past 2 years. The tool is also efficient in generating made-to-order proteins whose functions are yet to be discovered. Thus, industries and biomedical engineers are able to overcome the problems that were not solved earlier. This helps in designing user-friendly or personalized real-world proteins (structure and function) and apply them. The term deep learning has become common with the Baker's work *deep learning Protein MPBB platform*. In ML, the researchers give computer guidance, by which algorithm draws on the rich repository of real-world biological information to dream up new proteins based on the patterns and principles observed in nature. In some cases, DL also extracts additional features that are not obvious to scientists and hence drastically improving the performances and interpretability. The ML utilizes the conventional MS data, optimizes the number of true hits at a specified FDR and takes account of multiple peptide sequence features and experimental peptide data to re-score peptide in a semi-supervised manner. All these features are offered for learning and ML algorithm will automatically select and combine the most important ones. ML-generated information helps scientists to improve measurements. Percolator boosts the number of identified peptides by 5–16%, highlighting the power of ML-based method (Noor et al. 2021). AI or ML, DL and other tools have found wide applications in genomics and proteomics; however, the ethical and philosophical concerns rose concerning data quality; data privacy has raised challenges to stakeholders and authorities. Although FAIR principle is formalized, establishment of databases for sensitive data with restricted access has been suggested as common practice to overcome these hurdles (Grishin et al.

2019). The application of this advanced technology in fish "*-omics*" is yet to be visualized and practiced, which may be a turning point.

5.4 Conclusion

Current developments in genomics, transcriptomics, proteomics and metabolomics have opened innovative viewpoints for large-scale studies of genome- and proteome-wide patterns. Nevertheless, the proteomics is established in other organisms, this is still at an early stage in fish research, even as food. It would be a treasured tool for solving several queries in fisheries and aquaculture. It can be adopted for food quality, rapid seafood detection, targeted drug delivery, disease diagnosis, potential protein biomarkers of stress, reproduction, disease or the immune system and so on. Furthermore, an integrated approach of omics would aid in comprehending fish physiology and would be applied in fish farming/reproduction, health management and accurate seafood/species identification. In addition, the primary and secondary data derived would be advantageous in designing protein arrays, biosensors and microfluidic devices. Artificial intelligence would also leave its marks on the proteomics and the benefits of these would be immense ensuring better nutrition and health, and food quality and safety in a user-friendly manner.

References

Adkins JN, Varnum SM, Auberry KJ, Moore RJ, Angell NH, Smith RD, Pounds JG (2002) Toward a human blood serum proteome: analysis by multidimensional separation coupled with mass spectrometry. Mol Cell Proteomics 1(12):947–955

Alban A, David SO, Bjorkesten L, Andersson C, Sloge E, Lewis S, Currie I (2003) A novel experimental design for comparative two dimensional gel analysis: two dimensional difference gel electrophoresis incorporating a pooled internal standard. Proteomics 3(1):36–44

Aparicio S, Chapman J, Stupka E, Putnam N, Chia JM, Dehal P, Brenner S (2002) Whole-genome shotgun assembly and analysis of the genome of Fugu rubripes. Science 297(5585):1301–1310

Aslam B, Basit M, Nisar MA, Khurshid M, Rasool MH (2016) Proteomics: technologies and their applications. J Chromatogr Sci 55(2):182–196

Assefa A, Abunna F (2018) Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. Vet Med Int 2018:5432497

Banks RE, Dunn MJ, Hochstrasser DF, Sanchez JC, Blackstock W, Pappin DJ, Selby PJ (2000) Proteomics: new perspectives, new biomedical opportunities. Lancet 356(9243):1749–1756

Becker SA, Feist AM, Mo ML, Hannum G, Palsson BØ, Herrgard MJ (2007) Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox. Nat Protoc 2(3):727–738

Booy AT, Haddow JD, Ohlund LB, Hardie DB, Olafson RW (2005) Application of isotope coded affinity tag (ICAT) analysis for the identification of differentially expressed proteins following infection of Atlantic Salmon (Salmo salar) with infectious hematopoietic necrosis virus (IHNV) or Renibacterium salmoninarum (BKD). J Proteome Res 4(2):325–334

Canzler S, Schor J, Busch W, Schubert K, Rolle-Kampczyk UE, Seitz H, Hackermüller J (2020) Prospects and challenges of multi-omics data integration in toxicology. Arch Toxicol 94:371–388

Carr S, Aebersold R, Baldwin M, Burlingame AL, Clauser K, Nesvizhskii A (2004) The need for guidelines in publication of peptide and protein identification data: Working Group on Publication Guidelines for Peptide and Protein Identification Data. Mol Cell Proteomics 3(6):531–533

Carrera M, Piñeiro C, Martinez I (2020) Proteomic strategies to evaluate the impact of farming conditions on food quality and safety in aquaculture products. Food Secur 9(8):1050

Chandrasekhar K, Dileep A, Lebonah DE, Pramoda Kumari J (2014) A short review on proteomics and its applications. Int Lett Nat Sci 17:77–84

Chatr-Aryamontri A, Ceol A, Palazzi LM, Nardelli G, Schneider MV, Castagnoli L, Cesareni G (2007) MINT: the Molecular INTeraction database. Nucleic Acids Res 35(suppl_1):D572–D574

Chen G, Pramanik BN, Liu YH, Mirza UA (2007) Applications of LC/MS in structure identifications of small molecules and proteins in drug discovery. J Mass Spectrom 42(3):279–287

Chen ZT, Liang ZG, Zhu XD (2015) A review: proteomics in nasopharyngeal carcinoma. Int J Mol Sci 16(7):15497–15530

Chen C, Huang H, Wu CH (2017) Protein bioinformatics databases and resources. In: Protein bioinformatics: from protein modifications and networks to proteomics. Springer, New York, pp 3–39

Chiou SH, Wu CY (2011) Clinical proteomics: current status, challenges, and future perspectives. Kaohsiung J Med Sci 27(1):1–14

Ciereszko A, Dietrich MA, Nynca J (2017) Fish semen proteomics—new opportunities in fish reproductive research. Aquaculture 472:81–92

Croft D, O'kelly G, Wu G, Haw R, Gillespie M, Matthews L, Stein L (2010) Reactome: a database of reactions, pathways and biological processes. Nucleic Acids Res 39(1):D691–D697

Dalmasso E, Casenas D, Miller S (2009) Top-down, bottom-up: the merging of two high performance technologies. Bio-Rad Laboratories, Inc, Hercules

Damodaran S, Dlugos CA, Wood TD, Rabin RA (2006) Effects of chronic ethanol administration on brain protein levels: a proteomic investigation using 2-D DIGE system. Eur J Pharmacol 547(1-3):75–82

Decramer S, Wittke S, Mischak H, Zürbig P, Walden M, Bouissou F, Schanstra JP (2006) Predicting the clinical outcome of congenital unilateral ureteropelvic junction obstruction in newborn by urinary proteome analysis. Nat Med 12(4):398–400

Dietrich MA, Arnold GJ, Fröhlich T, Otte KA, Dietrich GJ, Ciereszko A (2015) Proteomic analysis of extracellular medium of cryopreserved carp (Cyprinus carpio L.) semen. Comp Biochem Physiol Part D 15:49–57

Domon B, Aebersold R (2006) Challenges and opportunities in proteomics data analysis. Mol Cell Proteomics 5(10):1921–1926

Forné I, Agulleiro MJ, Asensio E, Abián J, Cerdà J (2009) 2-D DIGE analysis of Senegalese sole (Solea senegalensis) testis proteome in wild-caught and hormone-treated F1 fish. Proteomics 9(8):2171–2181

Forné I, Abián J, Cerdà J (2010) Fish proteome analysis: model organisms and non-sequenced species. Proteomics 10(4):858–872

Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Jensen LJ (2012) STRING v9. 1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res 41(D1):D808–D815

Gebriel M, Prabhudesai S, Uleberg KE, Larssen E, Piston D, Bjørnstad AH, Møller SG (2014) Zebrafish brain proteomics reveals central proteins involved in neurodegeneration. J Neurosci Res 92(1):104–115

Glaab E, Baudot A, Krasnogor N, Schneider R, Valencia A (2012) EnrichNet: network-based gene set enrichment analysis. Bioinformatics 28(18):i451–i457

Gombar R, Pitcher TE, Lewis JA, Auld J, Vacratsis PO (2017) Proteomic characterization of seminal plasma from alternative reproductive tactics of Chinook salmon (Oncorhynchus tswatchysha). J Proteome 157:1–9

Graves PR, Haystead TA (2002) Molecular biologist's guide to proteomics. Microbiol Mol Biol Rev 66(1):39–63

Grishin D, Obbad K, Church GM (2019) Data privacy in the age of personal genomics. Nat Biotechnol 37(10):1115–1117
Gupta YR, Sellegounder D, Kannan M, Deepa S, Senthilkumaran B, Basavaraju Y (2016) Effect of copper nanoparticles exposure in the physiology of the common carp (Cyprinus carpio): Biochemical, histological and proteomic approaches. Aquac Fisheries 1:15–23
Han X, Jin M, Breuker K, McLafferty FW (2006) Extending top-down mass spectrometry to proteins with masses greater than 200 kDa. Science 314(5796):109–112
Hanash S (2003) Disease proteomics. Nature 422(6928):226–232
He Y, Huang H, Li LH, Yang X (2018) Label-free proteomics of tilapia fillets and their relationship with meat texture during post-mortem storage. Food Anal Methods 11:3023–3033
Hogstrand C, Balesaria S, Glover CN (2002) Application of genomics and proteomics for study of the integrated response to zinc exposure in a non-model fish species, the rainbow trout. Comp Biochem Physiol B: Biochem Mol Biol 133(4):523–535
Jaiswal S, Rasal KD, Chandra T, Prabha R, Iquebal MA, Rai A, Kumar D (2023) Proteomics in fish health and aquaculture productivity management: status and future perspectives. Aquaculture 566:739159
Jungbauer A, Hahn R (2009) Ion-exchange chromatography. Methods Enzymol 463:349–371
Kandasamy K, Mohan SS, Raju R, Keerthikumar S, Kumar GSS, Venugopal AK, Pandey A (2010) NetPath: a public resource of curated signal transduction pathways. Genome Biol 11:1–9
Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M (2012) KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids Res 40(D1):D109–D114
Kerrien S, Aranda B, Breuza L, Bridge A, Broackes-Carter F, Chen C, Hermjakob H (2012) The IntAct molecular interaction database in 2012. Nucleic Acids Res 40(D1):D841–D846
Kolder ICRM, Van Der Plas-Duivesteijn SJ, Tan G, Wiegertjes GF, Forlenza M, Guler AT, Spaink HP (2016) A full-body transcriptome and proteome resource for the European common carp. BMC Genomics 17:1–12
Komurov K, Ram PT (2010) Patterns of human gene expression variance show strong associations with signaling network hierarchy. BMC Syst Biol 4:1–12
König D, Oesser S, Scharla S, Zdzieblik D, Gollhofer A (2018) Specific collagen peptides improve bone mineral density and bone markers in postmenopausal women—a randomized controlled study. Nutrients 10(1):97
Krishnankutty R, Bhat A, Azmi A, Souchelnytskyi S, Uddin S (2016) An overview of proteomics techniques and its application as a tool in biomarker and drug discovery. J Proteomics Enzymol 2:2
Lahm HW, Langen H (2000) Mass spectrometry: a tool for the identification of proteins separated by gels. Electrophoresis 21(11):2105–2114
Lai CC, Tsai CH, Tsai FJ, Lee CC, Lin WD (2001) Rapid monitoring assay of congenital adrenal hyperplasia with microbore high-performance liquid chromatography/electrospray ionization tandem mass spectrometry from dried blood spots. Rapid Commun Mass Spectrom 15(22):2145–2151
Lecchi P, Gupte AR, Perez RE, Stockert LV, Abramson FP (2003) Size-exclusion chromatography in multidimensional separation schemes for proteome analysis. J Biochem Biophys Methods 56(1-3):141–152
Li L, Yu F, Zheng L, Wang R, Yan W, Wang Z, Jiang Q (2019) Natural hydrogels for cartilage regeneration: modification, preparation and application. J Orthop Transl 17:26–41
Li X, Feng C, Sha H, Zhou T, Zou G, Liang H (2022) Tandem mass tagging-based quantitative proteomics analysis reveals damage to the liver and brain of hypophthalmichthys molitrix exposed to acute hypoxia and reoxygenation. Antioxidants 11(3):589
Liu K, Li S, Wang L, Ye Y, Tang H (2020) Full-spectrum prediction of peptides tandem mass spectra using deep neural network. Anal Chem 92(6):4275–4283
López JL, Marina A, Álvarez G, Vázquez J (2002) Application of proteomics for fast identification of species-specific peptides from marine species. Proteomics 2(12):1658–1665

Ma Q, Liu X, Feng W, Liu S, Zhuang Z (2018) Analyses of the molecular mechanisms associated with salinity adaption of Trachidermus fasciatus through combined iTRAQ-based proteomics and RNA sequencing-based transcriptomics. Prog Biophys Mol Biol 136:40–53
Macek B, Waanders LF, Olsen JV, Mann M (2006) Top-down protein sequencing and MS3 on a hybrid linear quadrupole ion trap-orbitrap mass spectrometer. Mol Cell Proteomics 5(5):949–958
Macklin A, Khan S, Kislinger T (2020) Recent advances in mass spectrometry based clinical proteomics: applications to cancer research. Clin Proteomics 17:1–25
Mann M, Kumar C, Zeng WF, Strauss MT (2021) Artificial intelligence for proteomics and biomarker discovery. Cell Syst 12(8):759–770
Marouga R, David S, Hawkins E (2005) The development of the DIGE system: 2D fluorescence difference gel analysis technology. Anal Bioanal Chem 382:669–678
Martin DB, Nelson PS (2001) From genomics to proteomics: techniques and applications in cancer research. Trends Cell Biol 11(11):S60–S65
Martyniuk CJ, Denslow ND (2009) Towards functional genomics in fish using quantitative proteomics. Gen Comp Endocrinol 164(2-3):135–141
Metzker ML (2010) Sequencing technologies—the next generation. Nat Rev Genet 11(1):31–46
Mi H, Guo N, Kejariwal A, Thomas PD (2007) PANTHER version 6: protein sequence and function evolution data with expanded representation of biological pathways. Nucleic Acids Res 35(suppl_1):D247–D252
Mishra NC (2011) Introduction to proteomics: principles and applications. Wiley, Hoboken
Mohr J, Swart R, Samonig M, Böhm G, Huber CG (2010) High-efficiency nano-and micro-HPLC–high-resolution orbitrap-MS platform for top-down proteomics. Proteomics 10(20):3598–3609
Monti M, Cozzolino M, Cozzolino F, Tedesco R, Pucci P (2007) Functional proteomics: protein-protein interactions in vivo. Ital J Biochem 56(4):310–314
Moreira M, Schrama D, Farinha AP, Cerqueira M, Raposo de Magalhaes C, Carrilho R, Rodrigues P (2021) Fish pathology research and diagnosis in aquaculture of farmed fish; a proteomics perspective. Animals 11(1):125
Morro B, Doherty MK, Balseiro P, Handeland SO, MacKenzie S, Sveier H, Albalat A (2020) Plasma proteome profiling of freshwater and seawater life stages of rainbow trout (Oncorhynchus mykiss). PLoS One 15(1):e0227003
Moseley MA (2001) Current trends in differential expression proteomics: isotopically coded tags. Trends Biotechnol 19(10):S10–S16
Nesvizhskii AI, Keller A, Kolker E, Aebersold R (2003) A statistical model for identifying proteins by tandem mass spectrometry. Anal Chem 75(17):4646–4658
Nissa MU, Pinto N, Mukherjee A, Reddy PJ, Ghosh B, Sun Z, Srivastava S (2021a) Organ-based proteome and post-translational modification profiling of a widely cultivated tropical water fish, Labeo rohita. J Proteome Res 21(2):420–437
Nissa MU, Pinto N, Parkar H, Goswami M, Srivastava S (2021b) Proteomics in fisheries and aquaculture: an approach for food security. Food Control 127:108125
Nissa MU, Reddy PJ, Pinto N, Sun Z, Ghosh B, Moritz RL, Srivastava S (2022) The peptideatlas of a widely cultivated fish Labeo rohita: a resource for the aquaculture community. Sci Data 9(1):171
Noor Z, Ahn SB, Baker MS, Ranganathan S, Mohamedali A (2021) Mass spectrometry–based protein identification in proteomics—a review. Briefings in bioinformatics 22(2):1620–1638
Nuñez SM, Guzmán F, Valencia P, Almonacid S, Cárdenas C (2020) Collagen as a source of bioactive peptides: a bioinformatics approach. Electron J Biotechnol 48:101–108
Nynca J, Arnold GJ, Fröhlich T, Otte K, Flenkenthaler F, Ciereszko A (2014) Proteomic identification of rainbow trout seminal plasma proteins. Proteomics 14(1):133–140
Ong SE, Mann M (2005) Mass spectrometry–based proteomics turns quantitative. Nat Chem Biol 1(5):252–262
Ortea I, O'Connor G, Maquet A (2020) Review on proteomics for food authentication. J Proteome 147:212–225

Pampanin DM, Larssen E, Øysæd KB, Sundt RC, Sydnes MO (2014) Study of the bile proteome of Atlantic cod (Gadus morhua): multi-biological markers of exposure to polycyclic aromatic hydrocarbons. Mar Environ Res 101:161–168

Pan S, Aebersold R, Chen R, Rush J, Goodlett DR, McIntosh MW, Brentnall TA (2009) Mass spectrometry based targeted protein quantification: methods and applications. J Proteome Res 8(2):787–797

Pandey A, Mann M (2000) Proteomics to study genes and genomes. Nature 405(6788):837–846

Pastwa E, Somiari SB, Czyz M, Somiari RI (2007) Proteomics in human cancer research. Proteomics Clin Appl 1(1):4–17

Paulo JA (2013) Practical and efficient searching in proteomics: a cross engine comparison. Webmedcentral 4(10):52

Pineiro C, Barros-Velázquez J, Sotelo CG, Gallardo JM (1999) The use of two-dimensional electrophoresis in the characterization of the water-soluble protein fraction of commercial flat fish species. Z Lebensmittelunters Forschung A 208:342–348

Piras C, Roncada P, Rodrigues PM, Bonizzi L, Soggiu A (2016) Proteomics in food: quality, safety, microbes, and allergens. Proteomics 16(5):799–815

Reed MC, Nijhout HF, Neuhouser ML, Gregory JF III, Shane B, James SJ, Ulrich CM (2006) A mathematical model gives insights into nutritional and genetic aspects of folate-mediated one-carbon metabolism. J Nutr 136(10):2653–2661

Riffle M, Eng JK (2009) Proteomics data repositories. Proteomics 9(20):4653–4663

Rodrigues PM, Martin SA, Silva TS, Boonanuntanasarn S, Schrama D, Moreira M, Raposo C (2018) Proteomics in fish and aquaculture research. In: Proteomics in domestic animals: from farm to systems biology. Springer, Cham, pp 311–338

Rossel S, Barco A, Kloppmann M, Arbizu PM, Huwer B, Knebelsberger T (2021) Rapid species level identification of fish eggs by proteome fingerprinting using MALDI-TOF MS. J Proteome 231:103993

Rout MP, Aitchison JD, Suprapto A, Hjertaas K, Zhao Y, Chait BT (2000) The yeast nuclear pore complex: composition, architecture, and transport mechanism. J Cell Biol 148(4):635–652

Ruths D, Muller M, Tseng JT, Nakhleh L, Ram PT (2008) The signaling petri net-based simulator: a non-parametric strategy for characterizing the dynamics of cell-specific signaling networks. PLoS Comput Biol 4(2):e1000005

Saito M, Takenouchi Y, Kunisaki N, Kimura S (2001) Complete primary structure of rainbow trout type I collagen consisting of α1 (I) α2 (I) α3 (I) heterotrimers. Eur J Biochem 268(10):2817–2827

Saleh M, Kumar G, Abdel-Baki AA, Dkhil MA, El-Matbouli M, Al-Quraishy S (2018) Quantitative shotgun proteomics distinguishes wound-healing biomarker signatures in common carp skin mucus in response to Ichthyophthirius multifiliis. Vet Res 49:1–12

Saleh M, Kumar G, Abdel-Baki AAS, Dkhil MA, El-Matbouli M, Al-Quraishy S (2019) Quantitative proteomic profiling of immune responses to Ichthyophthirius multifiliis in common carp skin mucus. Fish Shellfish Immunol 84:834–842

Schaefer CF, Anthony K, Krupa S, Buchoff J, Day M, Hannay T, Buetow KH (2009) PID: the pathway interaction database. Nucleic Acids Res 37(suppl_1):D674–D679

Schmidt A, Forne I, Imhof A (2014) Bioinformatic analysis of proteomics data. BMC Syst Biol 8(2):1–7

Schmitz M, Ziv T, Admon A, Baekelandt S, Mandiki SN, L'Hoir M, Kestemont P (2017) Salinity stress, enhancing basal and induced immune responses in striped catfish Pangasianodon hypophthalmus (sauvage). J Proteome 167:12–24

Schwanhäusser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, Selbach M (2011) Global quantification of mammalian gene expression control. Nature 473(7347):337–342

Shen Y, Liu J, Estiu G, Isin B, Ahn YY, Lee DS, Oltvai ZN (2010) Blueprint for antimicrobial hit discovery targeting metabolic networks. Proc Natl Acad Sci 107(3):1082–1087

Sialana FJ, Schneebauer G, Paunkov A, Pelster B, Lubec G (2018) Proteomic studies on the swim bladder of the European eel (Anguilla anguilla). Proteomics 18(8):1700445

Smith L, Lind MJ, Welham KJ, Cawkwell L, Cancer Biology Proteomics Group (2006) Cancer proteomics and its application to discovery of therapy response markers in human cancer. Cancer 107(2):232–241

Tay TL, Lin Q, Seow TK, Tan KH, Hew CL, Gong Z (2006) Proteomic analysis of protein profiles during early development of the zebrafish, Danio rerio. Proteomics 6(10):3176–3188

Ulrich CM, Neuhouser M, Liu AY, Boynton A, Gregory JF III, Shane B, Nijhout HF (2008) Mathematical modeling of folate metabolism: predicted effects of genetic polymorphisms on mechanisms and biomarkers relevant to carcinogenesis. Cancer Epidemiol Biomarkers Prev 17(7):1822–1831

Vihinen M (2001) Bioinformatics in proteomics. Biomol Eng 18(5):241–248

Vizcaíno JA, Deutsch EW, Wang R, Csordas A, Reisinger F, Ríos D, Hermjakob H (2014) ProteomeXchange provides globally coordinated proteomics data submission and dissemination. Nat Biotechnol 32(3):223–226

Wang N, MacKenzie L, De Souza AG, Zhong H, Goss G, Li L (2007) Proteome profile of cytosolic component of zebrafish liver generated by LC− ESI MS/MS combined with trypsin digestion and microwave-assisted acid hydrolysis. J Proteome Res 6(1):263–272

Wang J, Luo D, Liang M, Zhang T, Yin X, Zhang Y, Liu W (2018) Spectrum-effect relationships between high-performance liquid chromatography (HPLC) fingerprints and the antioxidant and anti-inflammatory activities of collagen peptides. Molecules 23(12):3257

Wang KF, Wu YZ, Chi H (2023) A universal database reduction method based on the sequence tag strategy to facilitate large-scale database search in proteomics. Int J Mass Spectrom 483:116966

Weston AD, Hood L (2004) Systems biology, proteomics, and the future of health care: toward predictive, preventative, and personalized medicine. J Proteome Res 3(2):179–196

Wu R, Wu C, Liu D, Yang X, Huang J, Zhang J, He H (2018) Antioxidant and anti-freezing peptides from salmon collagen hydrolysate prepared by bacterial extracellular protease. Food Chem 248:346–352

Yadav SP (2007) The wholeness in suffix-omics, -omes, and the word om. J Biomol Tech 18(5):277

Yang M, Petralia F, Li Z, Li H, Ma W, Song X, Saez-Rodriguez J (2020) Community assessment of the predictability of cancer protein and phosphoprotein levels from genomics and transcriptomics. Cell Syst 11(2):186–195

Ye H, Lin Q, Luo H (2018) Applications of transcriptomics and proteomics in understanding fish immunity. Fish Shellfish Immunol 77:319–327

Yuan Z, Ye X, Hou Z, Chen S (2023) Sustainable utilization of proteins from fish processing by-products: extraction, biological activities and applications. Trends Food Sci Technol 2023:104276

Zhang L, Li Q, Hong H, Luo Y, Lametsch R (2020) Search for proteomic markers for stunning stress and stress-induced textural tenderization in silver carp (Hypophthalmichthys molitrix) fillets using label-free strategy. Food Res Int 137:109678

Part III

Food and Biomedical Applications

Food and Biomedical Applications of Fish Proteins

6

Maya Raman

Abstract

Fish-based diet and its health/nutritional benefits and global public awareness are on the rise. The fish protein and other bioactive compounds have innumerable health benefits for human well-being. Evidences suggest that proteins from fish have several beneficial metabolic effects. Fish-derived peptides containing bioactive amino acids beneficially influence pathways involved in body composition, hypertension, lipid profile, glucose metabolism, and many more. These also contain taurine, which is also known for its positive health benefits. Other than these benefits, proteins are also used in tissue engineering and other advanced therapeutical techniques. In this chapter, the food and biomedical applications of fish proteins are discussed in detail for the benefits of researchers, academicians, and industrialists.

Keywords

Food · Medical · Protein · Functional · Fish

6.1 Introduction

Proteins, documented as abundant source of nutrients essential for growth and development of the body and its metabolic activities, are referred to as essential biopolymers derived from different animal and plant sources. These are known for

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their outstanding functionalities and bioactivity (associated to its naturally active peptides) including health promotion. Therefore, proteins have gained considerable interest in different sectors including food processing and pharmaceuticals (French et al. 2016; Rehman et al. 2020; Khan et al. 2022).

Fish are rich source of proteins, and these play a very important role in terms of nutrition, economics, culture, and recreation (Lynch et al. 2016). This has also promoted the trade of fish and fishery products including fish proteins, polyunsaturated fatty acids (PUFA), micronutrients (vitamins such as, A, B, and D and minerals including calcium, selenium, zinc, iodine, and iron), and so on. Fish proteins aid in fighting malnutrition worldwide (Nyboer et al. 2019). Additionally, the demand for fish proteins is heightened due to the presence of the essential amino acids (methionine, tryptophan, threonine, cysteine, and lysine) and protein digestibility. Methionine and lysine, the limiting amino acids in terrestrial proteins, are found in high amounts in fish proteins (Tacon and Metian 2013). The demand for fish protein, being cheap source of protein and readily available, is exorbitantly increasing, and various culture and capture techniques are employed to make this sector sustainable.

The fish protein consumed by human contributes to about 40% and remaining 60% comprising of fish skin, head, frames, fins, trimmings, roes, viscera, and so on, are handsome source of protein that goes as underutilized waste or byproducts (Zamora-Sillero et al. 2018). These are generally utilized for the production of protein hydrolysates (Slizyte et al. 2016; Villamil et al. 2017; Lin et al. 2020). Fish proteins and peptides are known to exhibit anti-inflammatory, anticancer, antioxidant, and antihypertensive properties (Daud et al. 2015; Gavva et al. 2020; Hu et al. 2019). These also improve insulin sensitivity, regenerate skeletal muscles, enhance cell growth, and lower cardiovascular and metabolic syndrome risks including type-2 diabetes (Pilon et al. 2011; Tørris et al. 2016; De et al. 2020). And, on the other hand, among the functional properties, protein as a hydrocolloid is known for its emulsifying property, foaming ability, water holding capacity, stabilization and solubility, fat-binding ability, and gelling properties that make it significant as a functional additive in foods (Jemil et al. 2014; Cheetangdee 2017; Zhou et al. 2019; Phawaphuthanon et al. 2019). Fishery products including skin, muscle, viscera, and so on have other functional and commercial significance (Fig. 6.1).

6.1.1 Functional Attributes of Proteins and Peptides

6.1.1.1 Solubility

Solubility is a leading functional characteristics of proteins and protein hydrolysates, which is essential for emulsification and foaming (De et al. 2020). This is influenced by pH, which incompletely unbinds the protein and encourages conformational changes in the 3D structure (Tahergorabi et al. 2015). Peptides have greater solubility than parent proteins (He et al. 2013). The ionic and hydrophobic interactions interfere with the solubility. Hydrophobic interactions lead to protein coagulation, while ionic interactions enhance solubility by supporting protein-water interactions (O'Brien et al. 2007). The protein-solvent interactions are greater, when

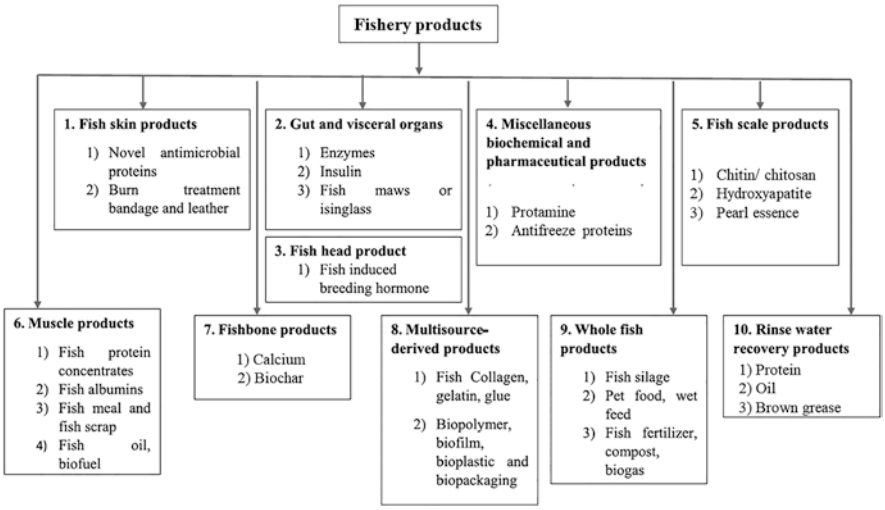

Fig. 6.1 Various functional and commercial applications of fishery products (Dale et al. 2019)

electrostatic repulsions are higher than hydrostatic interactions (Bauer et al. 2017). Other than pH, enzyme treatment, molecular weight, conformational conditions, surface characteristics of amino acids also influence the solubility (Haque et al. 2016).

6.1.1.2 Emulsifying Property

Emulsifying ability enhances the applications of proteins in emulsion-based food products (Abdollahi and Undeland 2018). Proteins are considered as excellent emulsifiers due to their amphipathic structure that promotes the absorption at the oil-water interface (Cardoso and Nunes 2013). Partial unfolding and denaturation is the major reason for high emulsifying property of peptides. The pH shift causes quick absorption of the hydrophobic spherical head of the treated proteins. The loss of tertiary structures causes conformational fluctuations at oil-water interface. The pH-shift process may also cause refolding; thus, offering support over native protein (Abaee et al. 2017). Emulsion stability index (ESI) of peptides could be improved by increasing pH. For instance, herring protein displayed supreme ESI at pH 11. It was reported that energetic sulfhydryl groups of peptides facilitate the formation of stable interface thin layers and ease the ESI. The emulsifying properties are influenced by pH, size, hydrophobicity, solubility, and surface charges of the proteins/peptides (Panpipat and Chaijan 2017).

6.1.1.3 Film-Forming Property

The synthetic packaging materials have created havoc on environment, and presently, to solve the issue, biodegradable packaging materials developed using biopolymers are gaining considerable interest. Natural polymers including polysaccharides, proteins, and lipids that are biocompatible and non-toxic, are

preferably used. The proteins are preferred for its enough mechanical and gas barrier properties and relative abundance. The pH, molecular weight, and solubility are some factors that together with intermolecular interactions contribute to the film forming ability of the proteins (Kaewprachu and Rawdkuen 2014; Lee et al. 2015).

6.1.1.4 Foaming Property

In certain food products such as whipped toppings, ice cream mixes, and baked foods, the volume and airspace are very critical, and foaming capability and foaming capacity of proteins make it suitable for this purposes (Lam et al. 2018). Foam capacity of protein is influenced by pH, whereby solubility is minimized at the isoelectric point (acidic or alkaline ends away from the isoelectric point). It was reported that the foaming capacity of protein significantly increases at alkaline pH (Yang and Baldwin 2017). The constancy of foams depends on the stability of the protein film at the gas-liquid interface and its absorbency capacity. Foam stabilizers also improve the foam stability and capacity (Kristinsson and Hultin 2003). Whey and egg form foams easily by reducing the surface tension. Similarly, the fact that the maximum foaming capability of fish protein hydrolysates at higher pH is due to the formation of solid, thick, and viscoelastic film formed by higher molecular weight protein aggregates (Panpipat and Chaijan 2017). Protein-based foams are mediated by transference, diffusion, and restructuring of molecules at the liquid/gas/interface, which may be modulated by modifying the physicochemical properties of the protein and its environment (Panpipat and Chaijan 2017).

6.1.1.5 Gelling Property

Fish muscle proteins contain structural proteins, namely myofibrillar protein and collagen, of which collagen is highly essential for its functional properties. Collagen is solubilized by dissolving in hot solution or heat-denaturation, which finds applications in food, cosmetics, and pharmaceuticals as a stabilizer (food additive), as it improves viscosity and gelling properties (Ahmad et al. 2017b). Gelatin (hydrolyzed collagen) are of two types: type A (acid hydrolyzed) and type B (alkali hydrolyzed), and as they exhibit dissimilar physicochemical properties; and these have multiple applications. Porcine skin and bovine hide are the raw material for commercial gelatin, but its applications find limitations due to religious and cultural reasons (Raman and Gopakumar 2018). Fish gelatin are used to produce edible films due its property to form thermoreversible gels (Raman and Gopakumar 2018). Fish gelatin could be a sustainable alternative to existing commercial sources. Several fishery by-products as membrane, fish bone, scales, swim bladder, and cartilages are used to produce the fish gelatin (Khan et al. 2022). However, the technical complications associated with fish gelatin are proline-hydroxyproline ratio, high production cost, variable quantity, and poor rheological behaviors (Tang et al. 2020). The low amounts of proline and hydroxyproline weaken the gelling strength of fish gelatin and hence, has lowest melting temperature. Other factors such as the molecular weight and complex interactions of amino acids and the proportion of α/β-chains also affect the gelling property (Sha et al. 2014). The gelling property

and functional attributes of fish gelatin may be improved by modulating these factors.

6.1.1.6 Water Holding Capacity

Proteins due to their unique chemical orientation have a strong water holding capacity (WHC); however, upon denaturation this is lost. This is evident in the case of myofibrillar proteins that denature during freezing and lose their hydrophilic property due to aggregation and loss of hydrogen bond formation (Agustini et al. 2008). For the flavor retention, texture, and mouthfeel, WHC plays an imperative role in the food systems. These can be improved by the additives such as carbohydrates. Fish protein isolates have higher WHC and OHP and thus have enhanced functional properties. Oil holding and water holding capacities are the hydrodynamic properties, significant for the classification of protein hydrolysates (Yu et al. 2018).

6.1.1.7 Oil Holding Property

Oil holding property (OHP) is mostly associated with enzyme-substrate specificity and emulsifying capacity and is influenced by bulk density and degree of hydrolysis (Villamil et al. 2017). OHP is significant in meat and confectionary products as it contributes to flavor (Taheri et al. 2013). The protein hydrolysis causes hydrophobicity, which is attributed to the non-polar residue of the proteins and the interactions (electrostatic, covalent, and hydrogen bond) (He et al. 2013; Mbatia et al. 2014). In blue-wing sea robin protein hydrolysate, a decline in lipid holding volume was observed, which was attributed to the increased molecular weight due to peptides (Sathivel et al. 2005). However, in other fish including salmon protein hydrolysates, the OHP was dependent on the enzyme-substrate ratio. The compromised stability of protein structure results in the degradation of protein networks formed due to entrapped oil. Oil holding capacity of many fish species have the abilities to be used as industrial oil binder in the processing of foods (Shaviklo and Johannsson 2006).

6.1.1.8 Digestibility

Fish muscle proteins are of significant importance for their superior nutritional quality, biological value, and bioavailability. The digestibility properties of fish proteins are also considerably high (Tahergorabi et al. 2015).

6.1.1.9 Color Characteristics

Color is a primary characteristic that strongly affects the consumer acceptance. Thermal processing may alter the color through various ways such as pigment formation or degradation, chemical reactions, oxidations, enzymatic/non-enzymatic browning, and coke formation (Ovissipour et al. 2013). Foh et al. (2012) suggested that higher brightness/redness is associated with native proteins and its stability. Kristinsson et al. (2007) and Rawdkuen et al. (2009) observed that at extreme acidic/alkaline pH, the myoglobin oxidizes resulting in high yellowness. Later, they also supported the view that these conditions quicken the auto-oxidation process.

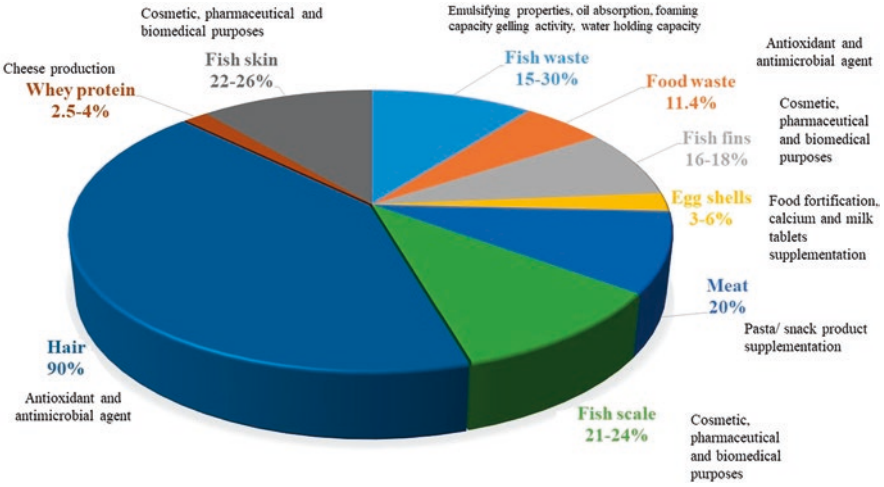

Fig. 6.2 Protein content and applications of various protein sources

6.1.1.10 Texture

This is a physical attribute associated with food matrix and its arrangements and the structure felt in the mouth (mouthfeel). Tensile strength, elongation at break, and puncture properties of proteins/peptides are some of the significant parameters that have an effect on the functionality of the end-products (Ovissipour et al. 2013).

These functional attributes also influence the organoleptic or sensory parameters of the proteins and peptides. Proteins influence the textural properties of the products, and hence, optimum ratio of proteins need to be taken into consideration during formulation.

Most common sources of protein for human consumption are fish and marine products (Petrova et al. 2018). In general, the protein content in fish vary from species to species and is also influenced by the other intrinsic and extrinsic factors and range between 8.2 and 23.9%, with high quantity being found in *Thunnus albacares* (23.9%) and least in *Harpodon nehereus* (8.2%). The concentration, quality, and type of protein differ from species to species (Raman and Mathew 2015). Different muscle types have different protein composition. The fish protein differs from lower vertebrates and land animals. For instance, no hemoglobin is found in mollusks. Similarly, there are variations among fish and mammalian derivative myoglobin composition; the former holds cysteine, while it is deficient in later. Figure 6.2 shows various protein sources and their applications.

6.2 Food Applications

Recent strategies in food technology are centered on the development of bio-based structures (specifically proteins and polysaccharides) for various applications including functional foods and nutraceuticals and food packaging. These structures

for food applications are generally recognized as safe (GRAS), biodegradable, biocompatible with suitable physicochemical and mechanical properties (Aditya et al. 2017). These structures do not induce inflammatory reactions, which are often associated with synthetic polymers (Martins et al. 2015). Macro-, micro-, and nanoscale bio-based structures are produced using proteins and polysaccharides. The design and applications of these structures depend on the functional features to be attributed to the final food products, which includes physicochemical stability, encapsulation properties, release characteristics, and rheological properties (Jones and McClements 2010).

Proteins have been extensively studied due to its characteristics such as solubility in water, biocompatibility, dispensability, and biodegradability. The industrial applications of proteins were known for decades. Normally, in food, proteins are directly added to improve its functionality such as gelling agent, emulsifier, and so on. (McClements et al. 2009; Lam and Nickerson 2013). The proteins may also be processed to form films, capsules, gels, foams, porous system, and fibers. Hence, there are numerous possibilities for the utilization of proteins as a functional material (Lefèvre et al. 2014).

Fish proteins and derived peptides were reported to be physiologically active or bioactive, either in their undisturbed form or after their release through hydrolysis (Fig. 6.3). More recently, proteins have been investigated as biomaterials for the bioactive compounds as a delivery vehicle for targeted delivery and release (Matalanis et al. 2011). Usually, bioactive compounds are vulnerable to food processing conditions and digestive environment; hence, to improve its bio-accessibility and bioavailability and changing food properties, proteins are studied (Martins et al.

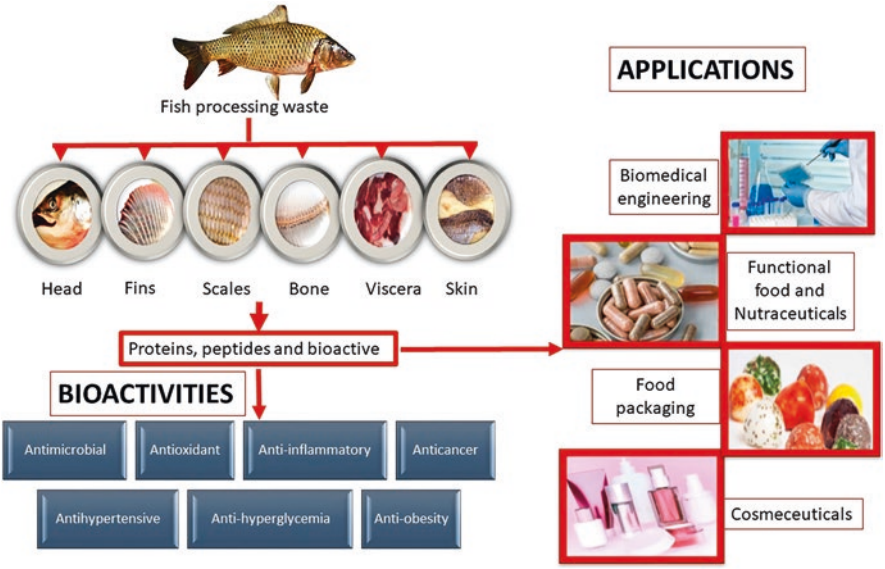

Fig. 6.3 Fish underutilized by-products and their applications and bioactivities

2015). Proteins are large biomolecules displaying high nutritional value, stabilizing effects, elasticity, and protective ability. These may be soluble or insoluble in water, acids, and bases. The properties exhibited by the proteins are due to its physico-chemical properties attributed by the amino acids, its quantity and sequence; and the interaction bonds available (McKee and McKee 2015; Gupta and Nayak 2015). The primary structure of the protein, susceptibility to enzymatic digestion, chemical changes during processing, amino acid composition, essential amino acid content are other significant parameters that effects its functionality. The hydrolyzed proteins or peptides, produced by gastrointestinal digestion or controlled enzymatic hydrolysis, have improved nutraceutical and bioactive properties over the parent protein. Peptides obtained from milk, cheese, eggs, meat, and fish proteins are widely deliberated and have been reported to possess antioxidant, antimicrobial, antihypertensive, and other bioactive properties (Pritchard et al. 2010; Meira et al. 2012).

6.3 Functional Foods and Nutraceuticals

The health potential of nutraceuticals and functional foods has not only triggered considerable interest from scientists and researchers worldwide but also effected the market that has shown a rapid growth. The rising consumer awareness, drastically changing life-style, and increasing prevalence of non-communicable diseases have impacted the nutraceutical and functional food market in a positive manner. Apart from being used to prevent or treat the non-communicable diseases, scientists are curious to identify the mechanism of action of these as it involves physiological interactions and interactions with molecules and food/drug, with themselves or with other constituents. Although the concept of functional food has been defined on several occasions, there is no internationally accepted definition for this term (Baker et al. 2022). Also, there is no scientific consensus to distinguish between functional foods, nutraceuticals, and dietary supplements. The market for nutraceuticals and functional food is increasing drastically every year, and the global functional food market is expected to reach $228.79 billion in 2025. The term "nutraceutical" was first coined by Stephen DeFelice, combining two words of "nutr*ition*"—*nourishing food or food component—and "pharm*aceuticals*"—referring to a drug for therapeutic activity* (Subramanian and Anandharamakrishnan 2023). Therefore, nutraceuticals and functional foods may be described as special category of additional bioactive components that serve specific purposes in human body, beyond basic nutrition. These prevent nutritional deficiencies, promote health and growth, and support development of the body, without adverse side-effects. Functional foods are known for potential anticancer, antioxidant, anti-inflammatory, and antidiabetic properties.

6.3.1 Fish Protein as a Functional Food/Nutraceutical and Its Effects on Health

Fish and fishery resources are excellent sources of high-quality proteins, and increasing amount of evidence points toward the beneficial effects of these on metabolic health (Aadland et al. 2016). Fish proteins contain all the essential amino acids and particularly have high amounts of lysine and leucine. It also contains aspartic acid, glutamic acid, and alanine. Taurine is also found in abundant amounts in fish protein (Ross et al. 2017). Furthermore, this protein has high digestibility (90%), making it highly exploitable. The fish protein is converted to protein hydrolysates for commercial applications. In the human gut, the whole protein undergoes enzymatic degradation (hydrolysis) or microbial fermentation to be converted to bioactive peptides (3–20 amino acids) (Ryan et al. 2011). Cod protein in combination with fish oil was reported to lower the rate of hepatic triacylglycerol secretion in-vivo; thus, altering lipid metabolism (De Moura et al. 2009). Similarly, cod protein in combination with soy proteins improved fasting glucose tolerance and peripheral insulin sensitivity possibly due to decreased release of pancreatic insulin and enhanced removal of insulin by the liver (De et al. 2020). These actions of cod protein were linked to the activity of amino acids on insulin-stimulated glucose uptake in skeletal muscle cells (Baker et al. 2022). The researchers linked insulin receptor substrate-1-associated phosphatidylinositol 3-kinase (PI3K)/Akt pathway that improved translocation of glucose transporter 4 to T-tubules in the cells, to the reduced insulin sensitivity. Therefore, the group emphasized that dietary cod proteins act as natural insulin-sensitizing agent (Divya and Jisha 2018). Lean seafood were also reported to be less obesogenic (Drotningsvik et al. 2018). Fish protein (cod, salmon) lowered weight gain, reduced visceral adiposity, and improved insulin sensitivity. Taurine also showed a negative correlation between adiposity. Together with glycine, it also exhibited anti-inflammatory effects (Garavand et al. 2022).

Several human intervention studies were also investigated to understand the effects of fish protein (Dale et al. 2019). Studies reported the beneficial effects of lean fish consumption on lipid status (Telle-Hansen et al. 2012; Aadland et al. 2015), glucose metabolism (Ouellet et al. 2008; Aadland et al. 2016), blood pressure (Erkkilä et al. 2008), and obesity (Ramel et al. 2009). The lean seafood protein from cod, pollock, saithe, and scallops showed promising results (Aadland et al. 2015). Not many studies intervened the beneficial effects of fish protein, except a study on the effects of cod protein on weight loss or weight management (Ramel et al. 2009). Salmon and cod proteins were reported to lower triacylglycerol levels in a 15-day parallel group intervention performed in 30 healthy individuals (Telle-Hansen et al. 2012). The salmon protein showed positive effects in reducing the triacylglycerol concentrations and increased the fasting levels of HDL-C in serum (Hagen et al. 2016). These results were again supported by another study in 33 patients with coronary heart diseases. Furthermore, fatty fish were reported to increase the particle size of HDL-C (Erkkilä et al. 2014). Nevertheless, the studies pertaining the effects of fish protein on humans are scarce. In premenopausal and postmenopausal women,

the lipid profiles were not much altered with the consumption of lead fish diet (Jacques et al. 1992; Gascon et al. 1996). In male subjects, these induced higher concentrations of plasma cholesterol and HDL-C, possibly due to the plasma sex hormone status and lipoprotein lipase activity (Lacaille et al. 2000). Lean seafood also altered the fasting and postprandial ratio of triacylglycerol to HDL-C (Aadland et al. 2016). Though cod protein showed neutral effects on cardiovascular health, it improved the insulin sensitivity and reduced the levels of circulating C-reactive proteins (Ouellet et al. 2008). Similarly, antihypertensive effects of seafood protein are minimal on human trials. A few studies indicated that lean fish reduce blood pressure in heart patients (Erkkilä et al. 2008).

6.3.2 Fish Protein Hydrolysate and Peptides as Functional Food/ Nutraceutical, and Its Health Effects

Several species of fish have been used to extract the protein hydrolysate (Fig. 6.4). The biological activity of the peptides has been attributed to the structural properties, molecular mass, and amino acid characteristics. Apart from these, the enzymes used for hydrolysis, pH, temperature, time taken for hydrolysis, and enzyme: substrate ratio are also significant. These bioactive peptides may have local effect in the gastrointestinal tract (GIT) or a systemic effect after absorption from gut and entering the circulation system (Ryan et al. 2011). Some amino acids are unique and contribute uniquely to the metabolic activities such as, reducing hypertension, inhibiting angiotensin-1 converting enzyme, altering blood glucose level, modulating gut microbiome, increasing bile acid conjugation, and so on (Le Gouic et al. 2018). Salmon collagen hydrolysate was reported to have antioxidant properties and antifreezing activities; hence, it may find applications as cryoprotectants in protein storage. The antioxidant activity of peptides was attributed to the presence of hydrophobic amino acids (valine and leucine) at N-terminus end and proline, histidine, or tyrosine in the sequences (Kristinsson and Rasco 2000). Further, the peptide fractions of salmon frame exhibited anti-inflammatory properties (Henaux et al. 2019). Antiallergic peptides from visceral hydrolysates of Atlantin salmon (*Salmo salar*) were also reported and could be a sustainable alternate (Wang et al. 2020).

Fish protein hydrolysates also exhibited cardio-protective effects in-vivo by reducing plasm total cholesterol, increasing high-density lipoprotein cholesterol (HDL-C), and lowering acyl-coenzyme A: cholesterol acyltransferase (ACAT) activity in the liver (Liaset et al. 2011). Similar effects were also reported for soy protein. These also reduced postprandial plasma glucose and triacylglycerol levels, resisting fatty diet–induced obesity. The plasma bile acid concentrations were elevated and were associated with molecular interventions. Saithe protein hydrolysate also exhibited similar pattern (Liaset et al. 2009). The high amounts of taurine and glycine in hydrolysate were reported to elevate fasting levels of bile acids and reduced visceral adipose tissue mass in-vivo (Liaset et al. 2009; Bjørndal et al. 2013). In another study, bioactive peptides in hydrolysates were reported to modulate the angiotensin-1 converting enzyme inhibition activities and beneficially alter

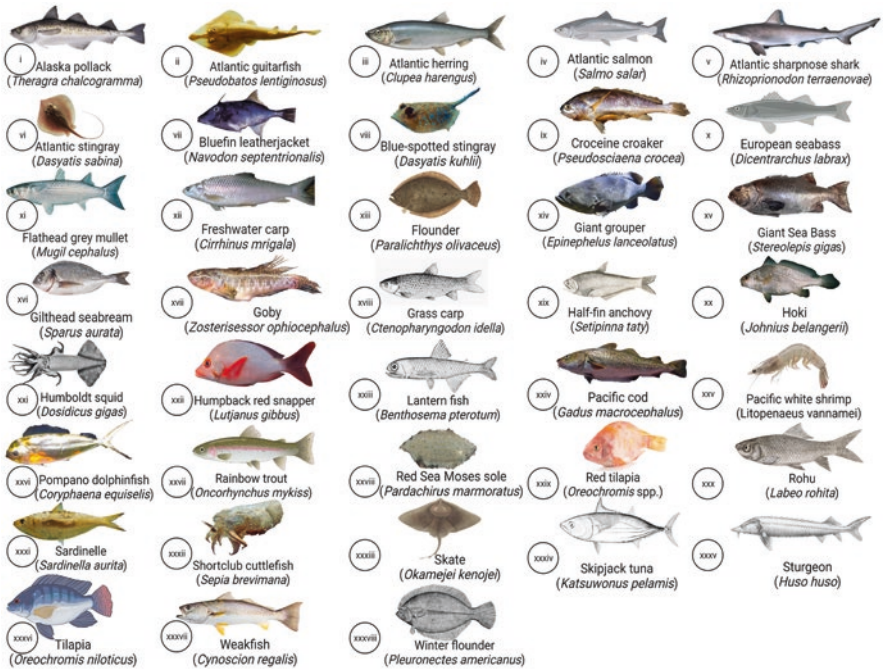

Fig. 6.4 Different fish species researched for obtaining protein hydrolysates. (Courtesy: Ramakrishnan et al. 2023)

Table 6.1 Fish protein hydrolysate and its functional applications in food industry and biomedicine

Sources	Functionality
Malaysian fish sausage (*Keropok lekor*) viscera, head, tail, frame	Antioxidant and antibacterial
Rainbow trout fish (*Oncorhynchus mykiss*) viscera	Food additive, excellent solubility, emulsifier, and foaming property Antioxidant and ACE inhibitory peptides
M. furnieri and *P. brasilensis*, fish muscle and skin	Antioxidant, biocompatible, release bioactive peptides
Cod fish (*Gadus morhua*) frames	Antioxidant, antihypertensive activity
Nile tilapia (*Oreochromis niloticus*) frame, dark muscle, skin, small bones and fins	Vascular function improvement in individuals with cardiovascular diseases
Lizard fish muscle	ACE inhibitory peptides released

the urine glucose, protein, and cystatin C concentrations (Drotningsvik et al. 2018) (Table 6.1).

Recent studies showed promising results upon investigating the effects of marine peptides as supplements for metabolic health in humans. Vikøren et al. (2013) were the first to investigate the specific effects of cod protein hydrolysate on metabolic

markets. The 8-week supplementation led to lowering of fasting and postprandial glucose levels by beneficially altering the postprandial insulin C-peptide concentrations. It also reduced body fat, increased lean body mass, and reduced LDL-C. However, in another study by same researchers on same protein showed no effects on insulin regulation, though non-esterified fatty acid concentrations in serum were reduced (Vildmyren et al. 2018). Sardine protein hydrolysate has significant antihypertensive effects by inhibiting ACE (Kawasaki et al. 2000). Similarly, protein hydrolysate was reported to have significantly reduced body weight, body mass index, and fat mass. It also reduced waist, thigh, and hip circumference (Nobile et al. 2016). Some studies also emphasized the importance of proteins in modulating the gut microbes, which upon dysbiosis lead to metabolic complications (Holm et al. 2016; Martinez et al. 2016).

The cod skin gelatin hydrolysates inhibited phosphorylated extracellular signal-regulated kinase (p-ERK), p-p38, and MMP-1 expression; and thus arrested collagen degradation. The peptides also inhibited phosphor-c-Jun N-terminal kinase (p-JNK) in the mitogen-activated protein kinase (MAPK) signaling pathway, which is involved in the photo-aging process. Therefore, these peptides could be used as a functional ingredient in the development of skin protection products (Lu et al. 2017). The squid peptides obtained from dried squid head also exhibited antioxidant activity and can be used in food (Ramakrishnan et al. 2023). The shrimp peptides (obtained from *Oratosquilla woodmasoni* and *Parapenaeus longirostris* waste) exhibited improved in-vitro ACE inhibition and antioxidant activity (Joshi et al. 2020; Messina et al. 2021), possibly attributed to the hydrophobic amino acids in the N-terminus end. These also contained proline and other aromatic groups in C-terminal end. Carotenoproteins from *M. affinis, Nematopalemon tenuipes, P. stylifera,* and *Penaeus monodon* shell waste serve as effective antioxidant. It may also be used as color enhancer. *P. stylifera* carotenoproteins were reported to be superior due to high quality of essential amino acids (Pattanaik et al. 2020). Low-molecular-weight peptides from *Solenocera crassicornis* heads were used to treat cyclophosphamide-induced hepatotoxicity (Jiang et al. 2020). Shrimp-derived peptides also exhibited memory protection (neuroprotective) and interacted with AChE (acetylcholinesterase) in-vivo (Wu et al. 2020). Hydrolysate obtained from *Litopenaeus seriferus* exhibited antioxidant activities and α-amylase inhibitory activity and inhibited lipid peroxidation (Kannan et al. 2011). Other than these applications, the fish protein/peptides also find several other industrial applications (Fig. 6.1). Food packaging is yet another aspect of food industry where fish proteins/peptides are utilized for their barrier properties.

6.3.3 Food Packaging: Edible Films/Coatings

The unwarranted use of fossil plastics has raised a greater global concern due to their high resistance to degradation and environmental issues due to the accumulation of its metabolites/by-products (Raza et al. 2018). The changing life-style, economic status, and consumption pattern of the world population have immensely

influenced this concern. Furthermore, the COVID-19 scenario had a significant mark on the food purchases by deliveries, which has also consequently increased the use of single-use plastic packaging (Adyel 2020; Kochańska et al. 2021). As a result, the size of the global plastic packaging market had 11.4% growth in revenue between 2019 and 2021, rising from $909.2 billion to $1012.6 billion (Business Insider 2020). These concerns have led scientists and industrialists to look for new avenues and sustainable materials for food packaging. Over the last decades, biodegradable films have been immensely investigated and pointed as a sustainable replacement for the petrochemical bases packaging materials (Asrofi et al. 2021). The bioplastics are usually derived from natural polymers such as starch (Marichelvam et al. 2019; Yang et al. 2020), cellulose (Azmin and Nor 2020), proteins (Jiménez-Rosado et al. 2020; Chalermthai et al. 2021; Mirpoor et al. 2022), and other materials (Yusmaniar et al. 2019; Lim et al. 2021) (Fig. 6.5). The biodegradable packaging materials and raw materials are identified; nevertheless, the challenges associated with edible packaging materials are production, storage and use of these ensuring consumer safety, integrity over time, and maintaining its functionality.

Proteins have proved to be highly suitable for producing biodegradable films among other biodegradable material because of their varied molecularity, good film-forming ability, and abundance. The efficiency of packaging films is also influenced by the biochemical changes on product surface that contribute to freshness and shelf stability. Proteins aid in encapsulation of food additives and biomolecules including flavors, antioxidants, antimicrobials, probiotics, and nanomaterials. Current interest of developing food packaging system is now focusing on protein-based materials that are safe, cost-effective, and sustainable. Figure 6.5 also depicts various protein sources used in developing food packaging system. The increased

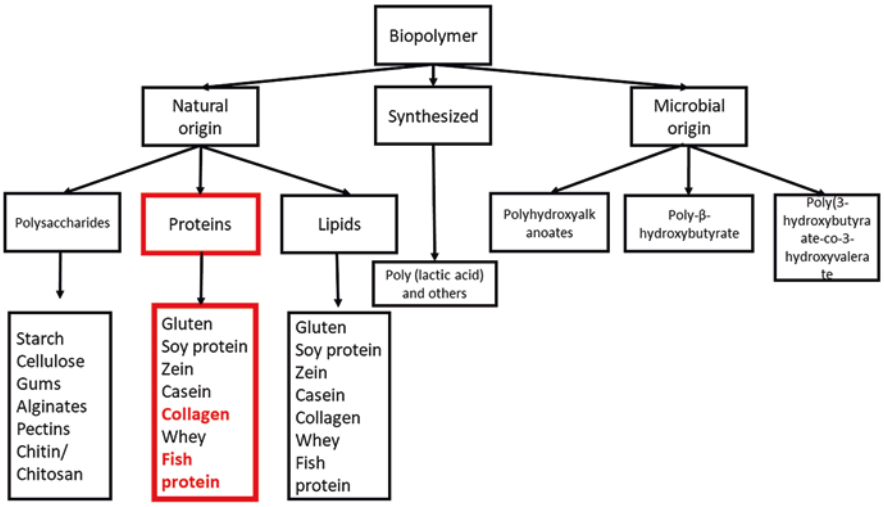

Fig. 6.5 Different types of biopolymers used in food packaging

attention to protein-based packaging materials is due to ease of formulation and processing and these being a renewable resource.

Proteins from fishery by-products have gained attention as innovative raw materials for developing bioplastics. These are of low cost and have the ability to form networks, plasticity, elasticity, and satisfactory oxygen barrier. Thus, it was studied immensely for film preparation (Kaewprachu et al. 2017)**.** Fish powder, an underutilized by-product of fish processing industry, was investigated for its applications as a biopolymer. It is generally generated during the filleting process, when frozen fish are filleted by a band-saw machine. Its composition indicates that it is a rich source of myofibrillar proteins. Usually these are discarded; however, Lima et al. (2022) suggested these as ideal material for developing biodegradable films. The fish-protein-based films generally have good barrier properties but unsatisfactory mechanical features including stiffness and malleability. Hence, it is combined with other biomaterials to overcome these challenges (La Fuente Arias et al. 2023). Other researches emphasized that myofibrillar protein are suitable for developing covering material as these are soluble in varying pH and insoluble in water (Wang et al. 2022). The pH of film-forming solution (FFS), plasticizers, preparing conditions, sources, and concentration of protein and materials added in FFS may affect the film properties (Florentino et al. 2022). Additionally, the concentration of FFS can affect the self-adhesion ability of mostly polymers, as well as the rate of polymer matrix forming in film suspensions could also be affected (Florentino et al. 2022). In another study, it has been found that the profound effect of different concentrations of fish myofibrillar protein (FMP) on the properties of films emphasizes that blue marlin film can be effectively prepared from 3% protein concentration. In packaging application, color is a significant indicator of films for customer commendation. The lightness, blueness/yellowness, and greenness/redness value of FMP films indicated that concentration has a direct effect on the color of FMP films (Du et al. 2021). Yellowish pigment formation generally occurs when film is formed at acidic condition. The FMP-based films remain intact for 24 h in aqueous system indicating the safety of the proteins. FMP films have film solubility in the range of 0.6–1.3%, whereas the petroleum-based polymeric films have poor water solubility. The solubility of FMP film is mostly influenced by the molecular weight of proteins than concentration (Kaewprachu et al. 2022). At high protein concentration, aggregation of polypeptide occurs resulting in maximum cross-linkages and greater molecular weight. Intermolecular covalent bonds intermingled in combination with hydrophobic or hydrogen items are the main forces involved during the film development. High solubility of film is an imperative feature of packaging materials (Arakawa et al. 2017). Table 6.2 shows the gist of various properties of muscle protein-derived polymer films found in existing literatures.

Food biopolymers exhibiting good barrier properties against gases, water vapor, moisture, and solutes are preferred for packaging, but these also find applications as food additives or as a carrier for antifungal, antioxidants, antimicrobials, colorants, and flavoring agents (Perera et al. 2023). Fish protein isolate (FPI)-based films are inflexible and have minimum water barrier property due to hydrophilicity of amino acid in protein molecules; however, the film flexibility is modified using suitable

Table 6.2 Properties of myofibrillar-based biopolymer films (Sarkar et al. 2023)

Source	Content	Thickness (mm)	Tensile strength (MPa)	Elongation (%)	Water vapor permeability (× 10–11 g/m/s/Pa)	Solubility (%)
Silver carp (*Hypophthalmichthys molitrix*)	Myofibrillar protein, glycerol, tannic acid	0.06	3.9	94	15	2
Tilapia (*Oreochromis niloticus*)	Myofibrillar protein, sorbitol	0.014	12.5	66	3.0	63
Pangasius boucourti fillet waste	Protein isolate	0.17	1.27	88	7.7	15
Whitemouth croaker (*Micropogonias furnieri*)	Myofibrillar protein	0.132	5.41	251	25	31
King weakfish (*Macrodon ancylodon*)	Myofibrillar protein, gelatin blend	0.106	6.5	384	2.7	27

amount of hydrophilic plasticizers. Trash dark muscle fish and yellow stripe trevally films were fabricated by mixing with fish skin gelatin (FSG) and FPI with the ratio of 1:1, having lower glycerol content (30%) at both pH (acidic and alkaline). The combination resulted in improved film properties due to the formation of hydrogen bond and other interactive bonds. Also, films exhibited great water barrier properties (Arfat et al. 2014).

Nanotechnology, a nano-based technique, is presently given more importance as it can improve the thermal, mechanical, and moisture repellent properties of biopolymer-based films (Ashfaq et al. 2022). As passive or traditional food packaging moved toward active/intelligent/innovative food packaging by incorporating nanotechnology, highly interactive, responsive, and functionally improved food packaging systems were investigated (Mlalila et al. 2016). Edible coatings with nanoparticles are more advantageous than conventional packaging materials in providing better preservation and food quality. The nanoparticles can modify the physical and mechanical properties of packaging polymer by improving their strength, durability, barrier, flexibility, and reusability properties (Bumbudsanpharoke et al. 2015). Biopolymers and inorganic materials in combination are generally used for improving the functional properties including antimicrobial, barrier properties, and so on (He et al. 2019). Table 6.3 shows different nanotechnology-based effective food packaging system.

The most studied fish by-products for nanoparticles are chitosan and fish proteins. Chitosan is a polysaccharide derived from chitin found in exoskeleton of crustaceans, arthropods, and cell walls of fungi. This is biocompatible, non-toxic, and environment friendly and exhibits excellent antimicrobial properties (due to chelating effects and ionic interactions) (Divya and Jisha 2018). Due to its hydrophobic nature and bond formation (hydrogen and covalent bonds), the moisture diffusion and permeability are modified considerably in films. Chitosan nanoparticles were reported to be effective in improving physical, mechanical, and barrier properties of bio-composite films (Garavand et al. 2022; Yanat and Schroën 2021). This is a novel area of research that has been gaining momentum in recent past. It could also find

Table 6.3 Nanoparticles used in food packaging in combination with proteins

Nanomaterial	Polymer matrix	Change in properties	Reference
Chitosan nanoparticles	Hydroxypropyl methylcellulose films reinforced with CNPs	Shelf life of plums and grapes increased	Shanmuga Priya et al. (2014)
	Hydroxypropyl methylcellulose edible films incorporated with chitosan/tripolyphosphate nanoparticles	Mechanical and barrier properties improved. Increase in thermal stability and water vapor permeability.	de Moura et al. (2009)
Nanoclay	Fish protein film incorporated with microbial transglutaminase and montmorillonite nanoclay	Water gain, water vapor permeability, solubility, tensile strength, elongation of nanocomposite improved	Rostamzad et al. (2016)

applications as active packaging material systems for food. Yet, this area of science needs further thorough investigations.

6.4 Biomedical Applications of Fish Protein

Biomaterials derived from various marine sources are employed to design for new generation biomedical applications. Several studies were conducted on natural sources including collagen, keratin, cellulose, hyaluronic acid, chitin, chitosan, pectin, calcium phosphate, biogenic silica, and so on. These naturally available biomaterials are readily available, ecofriendly, biocompatible, biodegradable, and cost-effective (Huang and Fu 2010; Flynn et al. 2007). Among these, marine-derived collagen and protein hydrolysates are in high demand. Marine collagen has more advantages than bovine collagen due to religious constraints in certain communities and immunogenic or inflammatory diseases possibly transmitted by the latter (Xu et al. 2021). These collagens contribute to bone and tissue health due to its rich amino acid and glycoprotein compositions (Ahmed et al. 2020). Similarly, protein hydrolysate derived from seafood processing waste not only reduces the environmental burden but also exhibits function and bioactive properties including antioxidative and antihypertensive activities (Petrova et al. 2018). Due to these reasons, these are utilized as functional foods also, as mentioned earlier.

6.4.1 Fish Skin–Derived Antimicrobial Proteins

The mucus found on fish surface were reported to have antimicrobial effects due to the proteins and enzymes present in it and the continuous production slough off the adhering microbes and parasites. The former properties are attributed to the presence of immunoglobin, complement proteins, lysozyme, C-reactive protein (CRP), lectins, proteolytic enzymes, transferrin, alkaline phosphatase, and other proteins and peptides (Subramanian et al. 2009; Arasu et al. 2013; Dash et al. 2018; Okella et al. 2021). Studies reported that these mucus have shown antimicrobial effects against *Staphylococcus aureus*, *Klebsiella penumoniae*, *Bacillus subtilis*, *Candida albicans, and Issatchenkia orientalis* (Hellio et al. 2002).

6.4.2 Wound Healing and Hydrogel

Due to the presence of type I collagen and ability to hold moisture, skin of several fish species are used for wound healing and as skin bandage. Studies indicate that tilapia skin has better mechanical properties and would withstand the stress and hence is used to treat second- and third-degree burns. The skin may be used as such after sterilization and storage under aseptic conditions (Lima-Junior et al. 2019). Song et al. (2006) demonstrated that collagen scaffolds could be derived from jellyfish. Absorbable surgical sutures that are essential for wound closing were

developed using fish gut collagen by Scientists of CIFT, Kerala (Maiti et al. 2000). Similar studies were also reported by other groups (Luan et al. 2022). Such kind of materials require high mechanical and biological performance, specifically in gastric conditions, where stomach bleeding hinders the normal healing process and deteriorates the condition. The collagen-based sutures developed using fish gut collagen exhibited resistance to acid and also intelligently sensed the stomach pH and act accordingly.

Hydrogels are also another form of artificial support that plays a key role in healing the soft tissues. Studies showed that swim bladder collagen from Bester sturgeon fish could be used to develop tough hydrogels (double network concept). This new class of collagen-based hybrid double network gels have good biomechanical performance and strong bonding ability with bone. These may be expanded to design next generation orthopedic implants including artificial cartilage, bone defect repair material (Mredha et al. 2017).

6.4.3 Fish Enzymes and Hormones

Fish enzymes are different from terrestrial sources with respect to amino acid composition, molecular weight, optimum pH and temperature, stability, and kinetic and inhibition characteristics (26). This is due to its adaptation to extreme environmental conditions including salinity, hydrostatic pressure, cold and hot water conditions, scarcity of limiting nutrients, oceanic waves, hydrothermal vents, and so on. The enzymes extracted from Antarctic fish exhibited thermosensitivity and high substrate specificity (Venugopal 2008). Furthermore, the enzymes derived from fish and shellfish may find multiple applications including pharmaceuticals, cosmeceuticals, leather processing, stick-water viscosity reduction, biosensors, gene cloning, and so on (Venugopal 2008; Borges et al. 2023).

Insulin (peptide hormone) in pure form may be easily extracted using fish gall bladder and bile duct, unlike the cattle insulin (Aditi and Varsha 2020). Insulin derived from fish are generally weaker due to amino acid sequence and immunological differences. It was reported that *Labeo rohita* bile attenuates hypoglycemic activity in streptozotocin-induced diabetes mellitus rats (Ahmad et al. 2017a).

6.4.4 Isinglass

Isinglass (fish maws) finds application in confectioneries and clarification of beverages. Apart from these, the isinglass is used as adhesive base, as binding agent in glass or pottery, in repairing leather belt, in textile sizing, and as ingredient in Indian ink (Flick and Martin 2000).

Other products include taurine, blood proteins, and antifreeze proteins that have gained scientific and technical interest among many potential users. Nevertheless, the studies pertaining to these are limited and need a thorough investigation. Among these, protamines have been commercialized, and salmon and herring sperm are

used to extract protamine. These are off-white crystalline or amorphous powder, colorless and odorless aqueous solution, used during delivery and heart surgery to neutralize heparin's anticoagulant effect (Haddadin and Faraday 2008). It is also used in combination with insulin to decrease the rate of absorption of insulin by tissues, thus prolonging the latter's effect (Windsor and Barlow 1981). Antifreeze peptides found in fish blood (Atlantic cod, white flounder, Atlantic wolfish, Greenland cod and sculpins) are colloidal glycopeptides with low freezing point (200–300 times more than salt) and is biocompatible. Therefore, it could find applications in frozen foods and dairy products as ice modulators (Venketesh and Dayananda 2008). Fish protein concentrate, gelatin, and collagen are other ingredients that also possess functional properties as mentioned earlier, which could find applications in food and pharmaceuticals.

6.5 Conclusion

Fish protein due to its unique composition and properties finds wide applications in food and pharmaceutical industries. Furthermore, the extraction of bioactives ensures environmental sustainability and eco-friendliness when these underutilized and fishery waste from processing sectors are used judiciously. The functional and nutraceutical applications of fish bioactive components are gaining considerable interest in recent years. Similarly, the biomedical applications are not lagging. However, there are numerous avenues that are still unexplored and need thorough research.

References

Aadland EK, Lavigne C, Graff IE, Eng Ø, Paquette M, Holthe A, Liaset B (2015) Lean-seafood intake reduces cardiovascular lipid risk factors in healthy subjects: results from a randomized controlled trial with a crossover design. Am J Clin Nutr 102(3):582–592

Aadland EK, Graff IE, Lavigne C, Eng Ø, Paquette M, Holthe A et al (2016) Lean seafood intake reduces postprandial C-peptide and lactate concentrations in healthy adults in a randomized controlled trial with a crossover design. J Nutr 146(5):1027–1034

Abaee A, Mohammadian M, Jafari SM (2017) Whey and soy protein-based hydrogels and nanohydrogels as bioactive delivery systems. Trends Food Sci Technol 70:69–81

Abdollahi M, Undeland I (2018) Structural, functional, and sensorial properties of protein isolate produced from salmon, cod, and herring by-products. Food Bioprocess Technol 11:1733–1749

Aditi M, Varsha L (2020) Fish processing: product and by-product, processing and marketing. Int J Fish Aquat Stud 8(1):74–77

Aditya NP, Espinosa YG, Norton IT (2017) Encapsulation systems for the delivery of hydrophilic nutraceuticals: food application. Biotechnol Adv 35(4):450–457

Adyel TM (2020) Accumulation of plastic waste during COVID-19. Science 369(6509):1314–1315

Agustini TW, Darmanto YS, Putri DPK (2008) Evaluation on utilization of small marine fish to produce surimi using different cryoprotective agents to increase the quality of surimi. J Coastal Dev 11(3):131–140

Ahmad D, Pottoo FH, Kumar RBS, Haldar S, Haldar PK (2017a) Rohu fish (*Labio rohita*) bile attenuates streptozotocin-induced diabetes mellitus in rats: evidence from biochemical and oxidative stress markers. Austin J Pharmacol Ther 5:1087

Ahmad T, Ismail A, Ahmad SA, Khalil KA, Kumar Y, Adeyemi KD, Sazili AQ (2017b) Recent advances on the role of process variables affecting gelatin yield and characteristics with special reference to enzymatic extraction: a review. Food Hydrocoll 63:85–96

Ahmed M, Verma AK, Patel R (2020) Collagen extraction and recent biological activities of collagen peptides derived from sea-food waste: a review. Sustain Chem Pharm 18:100315

Arakawa T, Ejima D, Akuta T (2017) Protein aggregation under high concentration/density state during chromatographic and ultrafiltration processes. Int J Biol Macromol 95:1153–1158

Arasu A, Kumaresan V, Sathyamoorthi A, Palanisamy R, Prabha N, Bhatt P et al (2013) Fish lily type lectin-1 contains β-prism architecture: immunological characterization. Mol Immunol 56(4):497–506

Arfat YA, Benjakul S, Prodpran T, Sumpavapol P, Songtipya P (2014) Properties and antimicrobial activity of fish protein isolate/fish skin gelatin film containing basil leaf essential oil and zinc oxide nanoparticles. Food Hydrocoll 41:265–273

Ashfaq A, Khursheed N, Fatima S, Anjum Z, Younis K (2022) Application of nanotechnology in food packaging: pros and cons. J Agric Food Res 7:100270

Asrofi M, Sapuan SM, Ilyas RA, Ramesh M (2021) Characteristic of composite bioplastics from tapioca starch and sugarcane bagasse fiber: effect of time duration of ultrasonication (Bath-Type). Materials Today Proc 46:1626–1630

Azmin SNHM, Nor MSM (2020) Development and characterization of food packaging bioplastic film from cocoa pod husk cellulose incorporated with sugarcane bagasse fibre. J Biores Bioprod 5(4):248–255

Baker MT, Lu P, Parrella JA, Leggette HR (2022) Consumer acceptance toward functional foods: a scoping review. Int J Environ Res Public Health 19(3):1217

Bauer KC, Hämmerling F, Kittelmann J, Dürr C, Görlich F, Hubbuch J (2017) Influence of structure properties on protein–protein interactions—QSAR modeling of changes in diffusion coefficients. Biotechnol Bioeng 114(4):821–831

Bjørndal B, Berge C, Ramsvik MS, Svardal A, Bohov P, Skorve J, Berge RK (2013) A fish protein hydrolysate alters fatty acid composition in liver and adipose tissue and increases plasma carnitine levels in a mouse model of chronic inflammation. Lipids Health Dis 12(1):1–11

Borges S, Odila J, Voss G, Martins R, Rosa A, Couto JA et al (2023) Fish by-products: a source of enzymes to generate circular bioactive hydrolysates. Molecules 28(3):1155

Bumbudsanpharoke N, Choi J, Ko S (2015) Applications of nanomaterials in food packaging. J Nanosci Nanotechnol 15(9):6357–6372

Business Insider (2020) COVID-19 impact on packaging market by material type, application and region—global forecast to 2021. Recovered from https://markets.businessinsider.com/news/stocks/covid-19-impact-on-packaging-market-by-material-type-application-and-region-global-forecast-to-2021–1029187123. Accessed 2 Dec 2021

Cardoso C, Nunes ML (2013) Improved utilization of fish waste, discards, and by-products and low-value fish towards food and health products. In: Utilization of fish waste, pp 26–58

Chalermthai B, Giwa A, Schmidt JE, Taher H (2021) Life cycle assessment of bioplastic production from whey protein obtained from dairy residues. Biores Technol Rep 15:100695

Cheetangdee N (2017) Characteristic of sausages as influenced by partial replacement of pork back-fat using pre-emulsified soybean oil stabilized by fish proteins isolate. Agric Nat Res 51(4):310–318

Dale HF, Madsen L, Lied GA (2019) Fish–derived proteins and their potential to improve human health. Nutr Rev 77(8):572–583

Dash S, Das SK, Samal J, Thatoi HN (2018) Epidermal mucus, a major determinant in fish health: a review. Iran J Vet Res 19(2):72

Daud NA, Babji AS, Yusop SM (2015) Effects of enzymatic hydrolysis on the antioxidative and antihypertensive activities from red tilapia fish protein. J Nutr Food Sci 5(387)

De Moura MR, Aouada FA, Avena-Bustillos RJ, McHugh TH, Krochta JM, Mattoso LH (2009) Improved barrier and mechanical properties of novel hydroxypropyl methylcellulose edible films with chitosan/tripolyphosphate nanoparticles. J Food Eng 92(4):448–453

De D, Sandeep KP, Kumar S, Raja RA, Mahalakshmi P, Sivaramakrishnan T et al (2020) Effect of fish waste hydrolysate on growth, survival, health of *Penaeus vannamei* and plankton diversity in culture systems. Aquaculture 524:735240

Divya K, Jisha MS (2018) Chitosan nanoparticles preparation and applications. Environ Chem Lett 16:101–112

Drotningsvik A, Pampanin DM, Slizyte R, Carvajal A, Høgøy I, Remman T, Gudbrandsen OA (2018) Hydrolyzed proteins from herring and salmon rest raw material contain peptide motifs with angiotensin-I converting enzyme inhibitors and resulted in lower urine concentrations of protein, cystatin C and glucose when fed to obese Zucker fa/fa rats. Nutr Res 52:14–21

Du H, Liu C, Unsalan O, Altunayar-Unsalan C, Xiong S, Manyande A, Chen H (2021) Development and characterization of fish myofibrillar protein/chitosan/rosemary extract composite edible films and the improvement of lipid oxidation stability during the grass carp fillets storage. Int J Biol Macromol 184:463–475

Erkkilä AT, Schwab US, de Mello VD, Lappalainen T, Mussalo H, Lehto S et al (2008) Effects of fatty and lean fish intake on blood pressure in subjects with coronary heart disease using multiple medications. Eur J Nutr 47:319–328

Erkkilä AT, Schwab US, Lehto S, deMello VD, Kangas AJ, Soininen P, Uusitupa MI (2014) Effect of fatty and lean fish intake on lipoprotein subclasses in subjects with coronary heart disease: a controlled trial. J Clin Lipidol 8(1):126–133

Flick GJ, Martin RE (2000) Industrial products: gelatin and isinglass production. In: Martin RE, Carter EP, Flick GJ, Davis LM Jr (eds) Marine and freshwater products handbook. Technomic Publishing Company Inc., Lancaster, pp 605–612

Florentino GIB, Lima DAS, Santos MMF, da Silva Ferreira VC, Grisi CVB, Madruga MS, da Silva FAP (2022) Characterization of a new food packaging material based on fish by-product proteins and passion fruit pectin. Food Packag Shelf Life 33:100920

Flynn L, Prestwich GD, Semple JL, Woodhouse KA (2007) Adipose tissue engineering with naturally derived scaffolds and adipose-derived stem cells. Biomaterials 28(26):3834–3842

Foh MBK, Wenshui X, Amadou I, Jiang Q (2012) Influence of pH shift on functional properties of protein isolated of tilapia (Oreochromis niloticus) muscles and of soy protein isolate. Food Bioprocess Technol 5:2192–2200

French KM, Somasuntharam I, Davis ME (2016) Self-assembling peptide-based delivery of therapeutics for myocardial infarction. Adv Drug Deliv Rev 96:40–53

Garavand F, Cacciotti I, Vahedikia N, Rehman A, Tarhan Ö, Akbari-Alavijeh S et al (2022) A comprehensive review on the nanocomposites loaded with chitosan nanoparticles for food packaging. Crit Rev Food Sci Nutr 62(5):1383–1416

Gascon A, Jacques H, Moorjani S, Deshaies Y, Brun LD, Julien P (1996) Plasma lipoprotein profile and lipolytic activities in response to the substitution of lean white fish for other animal protein sources in premenopausal women. Am J Clin Nutr 63(3):315–321

Gavva C, Patel K, Kudre T, Sharan K, Chilkunda DN (2020) Glycosaminoglycans from fresh water fish processing discard-isolation, structural characterization, and osteogenic activity. Int J Biol Macromol 145:558–567

Gupta P, Nayak KK (2015) Characteristics of protein-based biopolymer and its application. Polym Eng Sci 55(3):485–498

Haddadin AS, Faraday N (2008) Hemostasis, coagulopathy. In: Tamponade JVC, Baumgartner WA, Dorman T, Owens S (eds) The Johns Hopkins manual of cardiac surgical care, 2nd edn. Mosby, Elsevier Inc, Maryland, pp 237–256

Hagen IV, Helland A, Bratlie M, Brokstad KA, Rosenlund G, Sveier H et al (2016) High intake of fatty fish, but not of lean fish, affects serum concentrations of TAG and HDL-cholesterol in healthy, normal-weight adults: a randomised trial. Br J Nutr 116(4):648–657

Haque A, Timilsena YP, Adhikari B (2016) Food proteins, structure, and function. In: Reference module in food science, p 1À8

He S, Franco C, Zhang W (2013) Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). Food Res Int 50(1):289–297

He X, Deng H, Hwang HM (2019) The current application of nanotechnology in food and agriculture. J Food Drug Anal 27(1):1–21

Hellio C, Pons AM, Beaupoil C, Bourgougnon N, Le Gal Y (2002) Antibacterial, antifungal and cytotoxic activities of extracts from fish epidermis and epidermal mucus. Int J Antimicrob Agents 20(3):214–219

Henaux L, Thibodeau J, Pilon G, Gill T, Marette A, Bazinet L (2019) How charge and triple size-selective membrane separation of peptides from salmon protein hydrolysate orientate their biological response on glucose uptake. Int J Mol Sci 20(8):1939

Holm JB, Rønnevik A, Tastesen HS, Fjaere E, Fauske KR, Liisberg U et al (2016) Diet-induced obesity, energy metabolism and gut microbiota in C57BL/6J mice fed Western diets based on lean seafood or lean meat mixtures. J Nutr Biochem 31:127–136

Hu S, Yu J, Wang Y, Li Y, Chen H, Shi Y, Ma X (2019) Fish consumption could reduce the risk of oral cancer in Europeans: a meta-analysis. Arch Oral Biol 107:104494

Huang S, Fu X (2010) Naturally derived materials-based cell and drug delivery systems in skin regeneration. J Control Release 142(2):149–159

Jacques H, Noreau L, Moorjani S (1992) Effects on plasma lipoproteins and endogenous sex hormones of substituting lean white fish for other animal-protein sources in diets of postmenopausal women. Am J Clin Nutr 55(4):896–901

Jemil I, Jridi M, Nasri R, Ktari N, Salem RBSB, Mehiri M et al (2014) Functional, antioxidant and antibacterial properties of protein hydrolysates prepared from fish meat fermented by *Bacillus subtilis* A26. Process Biochem 49(6):963–972

Jiang S, Zhang Z, Yu F, Zhang Z, Yang Z, Tang Y, Ding G (2020) Ameliorative effect of low molecular weight peptides from the head of red shrimp (*Solenocera crassicornis*) against cyclophosphamide-induced hepatotoxicity in mice. J Funct Foods 72:104085

Jiménez-Rosado M, Bouroudian E, Perez-Puyana V, Guerrero A, Romero A (2020) Evaluation of different strengthening methods in the mechanical and functional properties of soy protein-based bioplastics. J Clean Prod 262:121517

Jones OG, McClements DJ (2010) Functional biopolymer particles: design, fabrication, and applications. Compr Rev Food Sci Food Saf 9(4):374–397

Joshi I, Janagaraj K, Nazeer RA (2020) Isolation and characterization of angiotensin I-converting enzyme (ACE-I) inhibition and antioxidant peptide from by-catch shrimp (Oratosquilla woodmasoni) waste. Biocatal Agric Biotechnol 29:101770

Kaewprachu P, Rawdkuen S (2014) Mechanical and physico-chemical properties of biodegradable protein-based films: a comparative study. Food Appl Biosci J 2(1):15–30

Kaewprachu P, Osako K, Tongdeesoontorn W, Rawdkuen S (2017) The effects of microbial transglutaminase on the properties of fish myofibrillar protein film. Food Packag Shelf Life 12:91–99

Kaewprachu P, Osako K, Rungraeng N, Rawdkuen S (2022) Properties of fish myofibrillar protein film: effect of glycerol-sorbitol combinations. J Food Sci Technol 59(4):1619–1628

Kannan A, Hettiarachchy NS, Marshall M, Raghavan S, Kristinsson H (2011) Shrimp shell peptide hydrolysates inhibit human cancer cell proliferation. J Sci Food Agric 91(10):1920–1924

Kawasaki T, Seki E, Osajima K, Yoshida M, Asada K, Matsui T, Osajima Y (2000) Antihypertensive effect of valyl-tyrosine, a short chain peptide derived from sardine muscle hydrolyzate, on mild hypertensive subjects. J Hum Hypertens 14(8):519–523

Khan S, Rehman A, Shah H, Aadil RM, Ali A, Shehzad Q et al (2022) Fish protein and its derivatives: the novel applications, bioactivities, and their functional significance in food products. Food Rev Intl 38(8):1607–1634

Kochańska E, Łukasik RM, Dzikuć M (2021) New circular challenges in the development of take-away food packaging in the COVID-19 period. Energies 14(15):4705

Kristinsson HG, Hultin HO (2003) Effect of low and high pH treatment on the functional properties of cod muscle proteins. J Agric Food Chem 51(17):5103–5110

Kristinsson HG, Rasco BA (2000) Biochemical and functional properties of Atlantic salmon (Salmo salar) muscle proteins hydrolyzed with various alkaline proteases. J Agric Food Chem 48(3):657–666

Kristinsson HG, Theodore AE, Ingadottir B (2007) Chemical processing methods for protein recovery from marine by-products and underutilized fish species. In: Maximising the value of marine by-products. Woodhead Publishing, pp 144–168

La Fuente Arias CI, Kubo MTKN, Tadini CC, Augusto PED (2023) Bio-based multilayer films: a review of the principal methods of production and challenges. Crit Rev Food Sci Nutr 63(14):2260–2276

Lacaille B, Julien P, Deshaies Y, Lavigne C, Brun LD, Jacques H (2000) Responses of plasma lipoproteins and sex hormones to the consumption of lean fish incorporated in a prudent-type diet in normolipidemic men. J Am Coll Nutr 19(6):745–753

Lam RS, Nickerson MT (2013) Food proteins: a review on their emulsifying properties using a structure–function approach. Food Chem 141(2):975–984

Lam ACY, Can Karaca A, Tyler RT, Nickerson MT (2018) Pea protein isolates: structure, extraction, and functionality. Food Rev Intl 34(2):126–147

Le Gouic AV, Harnedy PA, FitzGerald RJ (2018) Bioactive peptides from fish protein by-products. In: Bioactive molecules in food, pp 1–35

Lee JH, Lee JH, Yang HJ, Won M, Song KB (2015) Characterisation of jellyfish protein films with added transglutaminase and wasabi extract. Int J Food Sci Technol 50(7):1683–1689

Lefèvre T, Byette F, Marcotte I, Auger M (2014) Protein-and peptide-based materials: a source of inspiration for innovation. In: Leclerc M, Gauvin R (eds) Functional materials–for energy, sustainable development and biomedical sciences. De Gruyter, Berlin, pp 415–442

Liaset B, Madsen L, Hao Q, Criales G, Mellgren G, Marschall HU et al (2009) Fish protein hydrolysate elevates plasma bile acids and reduces visceral adipose tissue mass in rats. BBA-Mol Cell Biol Lipids 1791(4):254–262

Liaset B, Hao Q, Jørgensen H, Hallenborg P, Du ZY, Ma T et al (2011) Nutritional regulation of bile acid metabolism is associated with improved pathological characteristics of the metabolic syndrome. J Biol Chem 286(32):28382–28395

Lim C, Yusoff S, Ng CG, Lim PE, Ching YC (2021) Bioplastic made from seaweed polysaccharides with green production methods. J Environ Chem Eng 9(5):105895

Lima DAS, Santos MMF, Sousa AMBL, Bezerra TKA, da Silva Araújo ÍB, Madruga MS, da Silva FAP (2022) The cutting by-product of fish filleting on the band saw machine: nutritional quality and technological potential. Waste Biomass Valori 13(11):4575–4584

Lima-Junior EM, de Moraes Filho MO, Costa BA, Fechine FV, de Moraes MEA, Silva-Junior FR et al (2019) Innovative treatment using tilapia skin as a xenograft for partial thickness burns after a gunpowder explosion. J Surg Case Rep 2019(6):rjz181

Lin Y, Cai X, Wu X, Lin S, Wang S (2020) Fabrication of snapper fish scales protein hydrolysate-calcium complex and the promotion in calcium cellular uptake. J Funct Foods 65:103717

Lu J, Hou H, Fan Y, Yang T, Li B (2017) Identification of MMP-1 inhibitory peptides from cod skin gelatin hydrolysates and the inhibition mechanism by MAPK signaling pathway. J Funct Foods 33:251–260

Luan Z, Liu S, Wang W, Xu K, Ye S, Dan R et al (2022) Aligned nanofibrous collagen membranes from fish swim bladder as a tough and acid-resistant suture for pH-regulated stomach perforation and tendon rupture. Biomat Res 26(1):60

Lynch AJ, Cooke SJ, Deines AM, Bower SD, Bunnell DB, Cowx IG et al (2016) The social, economic, and environmental importance of inland fish and fisheries. Environ Rev 24(2):115–121

Maiti SK, Hoque M, Kumar N, Singh GR (2000) A new absorbable suture from fish-gut collagen-an experimental study. Indian Vet J 77(5):405–407

Marichelvam MK, Jawaid M, Asim M (2019) Corn and rice starch-based bio-plastics as alternative packaging materials. Fibers 7(4):32

Martinez KB, Pierre JF, Chang EB (2016) The gut microbiota: the gateway to improved metabolism. Gastroenterol Clin 45(4):601–614

Martins JT, Ramos OL, Pinheiro AC, Bourbon AI, Silva HD, Rivera MC et al (2015) Edible bio-based nanostructures: delivery, absorption and potential toxicity. Food Eng Rev 7:491–513

Matalanis A, Jones OG, McClements DJ (2011) Structured biopolymer-based delivery systems for encapsulation, protection, and release of lipophilic compounds. Food Hydrocoll 25(8):1865–1880

Mbatia B, Ogonda LA, Muge EK, Mulaa FJ (2014) Antioxidative and functional properties of *Rastrineobola argentea* (dagaa) fish protein hydrolysate. Discourse JAFS 2(6):180–189

McClements DJ, Decker EA, Park Y, Weiss J (2009) Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. Crit Rev Food Sci Nutr 49(6):577–606

McKee T, McKee JR (2015) Amino acids, peptides, and proteins. In: McKee T, McKee JR (eds) Biochemistry: the molecular basis of life, 5th edn. Oxford University Press, New York, pp 123–181

Meira SMM, Daroit DJ, Helfer VE, Corrêa APF, Segalin J, Carro S, Brandelli A (2012) Bioactive peptides in water-soluble extracts of ovine cheeses from Southern Brazil and Uruguay. Food Res Int 48(1):322–329

Messina CM, Manuguerra S, Arena R, Renda G, Ficano G, Randazzo M et al (2021) In vitro bioactivity of astaxanthin and peptides from hydrolisates of shrimp (*Parapenaeus longirostris*) by-products: from the extraction process to biological effect evaluation, as pilot actions for the strategy "from waste to profit". Mar Drugs 19(4):216

Mirpoor SF, Varriale S, Porta R, Naviglio D, Spennato M, Gardossi L et al (2022) A biorefinery approach for the conversion of *Cynara cardunculus* biomass to active films. Food Hydrocoll 122:107099

Mlalila N, Kadam DM, Swai H, Hilonga A (2016) Transformation of food packaging from passive to innovative via nanotechnology: concepts and critiques. J Food Sci Technol 53:3395–3407

Mredha MTI, Kitamura N, Nonoyama T, Wada S, Goto K, Zhang X et al (2017) Anisotropic tough double network hydrogel from fish collagen and its spontaneous in vivo bonding to bone. Biomaterials 132:85–95

Nobile V, Duclos E, Michelotti A, Bizzaro G, Negro M, Soisson F (2016) Supplementation with a fish protein hydrolysate (Micromesistius poutassou): effects on body weight, body composition, and CCK/GLP-1 secretion. Food Nutr Res 60(1):29857

Nyboer EA, Liang C, Chapman LJ (2019) Assessing the vulnerability of Africa's freshwater fishes to climate change: a continent-wide trait-based analysis. Biol Conserv 236:505–520

O'Brien EP, Dima RI, Brooks B, Thirumalai D (2007) Interactions between hydrophobic and ionic solutes in aqueous guanidinium chloride and urea solutions: lessons for protein denaturation mechanism. J Am Chem Soc 129(23):7346–7353

Okella H, Ikiriza H, Ochwo S, Ajayi CO, Ndekezi C, Nkamwesiga J et al (2021) Identification of antimicrobial peptides isolated from the skin mucus of African catfish, *Clarias gariepinus* (Burchell, 1822). Front Microbiol 12:794631

Ouellet V, Weisnagel SJ, Marois J, Bergeron J, Julien P, Gougeon R et al (2008) Dietary cod protein reduces plasma C-reactive protein in insulin-resistant men and women. J Nutr 138(12):2386–2391

Ovissipour M, Rasco B, Tang J, Sablani SS (2013) Kinetics of quality changes in whole blue mussel (*Mytilus edulis*) during pasteurization. Food Res Int 53(1):141–148

Panpipat W, Chaijan M (2017) Functional properties of pH-shifted protein isolates from bigeye snapper (*Priacanthus tayenus*) head by-product. Int J Food Prop 20(3):596–610

Pattanaik SS, Sawant PB, Xavier KM, Dube K, Srivastava PP, Dhanabalan V, Chadha NK (2020) Characterization of carotenoprotein from different shrimp shell waste for possible use as supplementary nutritive feed ingredient in animal diets. Aquaculture 515:734594

Perera KY, Jaiswal AK, Jaiswal S (2023) Biopolymer-based sustainable food packaging materials: challenges, solutions, and applications. Foods 12(12):2422

Petrova I, Tolstorebrov I, Eikevik TM (2018) Production of fish protein hydrolysates step by step: technological aspects, equipment used, major energy costs and methods of their minimizing. Int Aqua Res 10:223–241

Phawaphuthanon N, Yu D, Ngamnikom P, Shin IS, Chung D (2019) Effect of fish gelatine-sodium alginate interactions on foam formation and stability. Food Hydrocoll 88:119–126

Pilon G, Ruzzin J, Rioux LE, Lavigne C, White PJ, Frøyland L et al (2011) Differential effects of various fish proteins in altering body weight, adiposity, inflammatory status, and insulin sensitivity in high-fat–fed rats. Metabolism 60(8):1122–1130

Pritchard SR, Phillips M, Kailasapathy K (2010) Identification of bioactive peptides in commercial cheddar cheese. Food Res Int 43(5):1545–1548

Ramakrishnan SR, Jeong CR, Park JW, Cho SS, Kim SJ (2023) A review on the processing of functional proteins or peptides derived from fish by-products and their industrial applications. Heliyon 9:e14188

Raman M, Gopakumar K (2018) Fish collagen and its applications in food and pharmaceutical industry: a review. EC Nutrit 13:752–767

Raman M, Mathew S (2015) Physiochemical and textural alterations in Indian squid (*Loligo duvauceli*) mantle during frozen storage and cooking. J Aqua Food Prod Technol 24(5):454–467

Ramel A, Jonsdottir MT, Thorsdottir I (2009) Consumption of cod and weight loss in young overweight and obese adults on an energy reduced diet for 8-weeks. Nutr Metab Cardiovasc Dis 19(10):690–696

Rawdkuen S, Sai-Ut S, Khamsorn S, Chaijan M, Benjakul S (2009) Biochemical and gelling properties of tilapia surimi and protein recovered using an acid-alkaline process. Food Chem 112(1):112–119

Raza A, Ghafoor S, Butt MFU (2018) MIMO-enabled integrated MGDM–WDM distributed antenna system architecture based on plastic optical fibers for millimeter-wave communication. Photon Netw Commun 35:265–273

Rehman A, Tong Q, Jafari SM, Assadpour E, Shehzad Q, Aadil RM et al (2020) Carotenoid-loaded nanocarriers: a comprehensive review. Adv Colloid Interf Sci 275:102048

Ross A, Vincent A, Savolainen OI, Sandberg AS, Undeland I (2017) Dietary protein sources beyond proteins and amino acids-a comparative study of the small molecular weight components of meat and fish using metabolomics. FASEB J 31:652–613

Rostamzad H, Paighambari SY, Shabanpour B, Ojagh SM, Mousavi SM (2016) Improvement of fish protein film with nanoclay and transglutaminase for food packaging. Food Packag Shelf Life 7:1–7

Ryan JT, Ross RP, Bolton D, Fitzgerald GF, Stanton C (2011) Bioactive peptides from muscle sources: meat and fish. Nutrients 3(9):765–791

Sarkar MSI, Hasan MM, Hossain MS, Khan M, Al Islam A, Paul SK et al (2023) Exploring fish in a new way: a review on non-food industrial applications of fish. Heliyon 9:e22673

Sathivel S, Smiley S, Prinyawiwatkul W, Bechtel PJ (2005) Functional and nutritional properties of red salmon (*Oncorhynchus nerka*) enzymatic hydrolysates. J Food Sci 70(6):c401–c406

Sha XM, Tu ZC, Liu W, Wang H, Shi Y, Huang T, Man ZZ (2014) Effect of ammonium sulfate fractional precipitation on gel strength and characteristics of gelatin from bighead carp (*Hypophthalmichthys nobilis*) scale. Food Hydrocoll 36:173–180

Shanmuga Priya D, Suriyaprabha R, Yuvakkumar R, Rajendran V (2014) Chitosan-incorporated different nanocomposite HPMC films for food preservation. J Nanopart Res 16:1–16

Shaviklo GR, Johannsson SR (2006) Quality assessment of fish protein isolates using surimi standard methods. Fisheries Training Programme, Final Project

Slizyte R, Rommi K, Mozuraityte R, Eck P, Five K, Rustad T (2016) Bioactivities of fish protein hydrolysates from defatted salmon backbones. Biotechnol Rep 11:99–109

Song E, Kim SY, Chun T, Byun HJ, Lee YM (2006) Collagen scaffolds derived from a marine source and their biocompatibility. Biomaterials 27(15):2951–2961

Subramanian P, Anandharamakrishnan C (2023) Introduction to functional foods and nutraceuticals. In: Industrial application of functional foods, ingredients and nutraceuticals. Academic Press, pp 3–43

Subramanian S, Ross NW, MacKinnon SL (2009) Myxinidin, a novel antimicrobial peptide from the epidermal mucus of hagfish, *Myxine glutinosa* L. Mar Biotechnol 11:748–757

Tacon AG, Metian M (2013) Fish matters: importance of aquatic foods in human nutrition and global food supply. Rev Fish Sci 21(1):22–38

Tahergorabi R, Matak KE, Jaczynski J (2015) Fish protein isolate: development of functional foods with nutraceutical ingredients. J Funct Foods 18:746–756
Taheri A, Anvar SAA, Ahari H, Fogliano V (2013) Comparison the functional properties of protein hydrolysates from poultry by-products and rainbow trout (*Onchorhynchus mykiss*) viscera. Iran J Fish Sci
Tang M, Dai H, Ma L, Yu Y, Liu T, Feng X et al (2020) Degradation of structural proteins and their relationship with the quality of Mandarin fish (*Siniperca chuatsi*) during post-mortem storage and cooking. Int J Food Sci Technol 55(4):1617–1628
Telle-Hansen VH, Larsen LN, Høstmark AT, Molin M, Dahl L, Almendingen K, Ulven SM (2012) Daily intake of cod or salmon for 2 weeks decreases the 18: 1n-9/18: 0 ratio and serum triacylglycerols in healthy subjects. Lipids 47:151–160
Tørris C, Molin M, Cvancarova MS (2016) Lean fish consumption is associated with lower risk of metabolic syndrome: a Norwegian cross sectional study. BMC Public Health 16:1–9
Venketesh S, Dayananda C (2008) Properties, potentials, and prospects of antifreeze proteins. Crit Rev Biotechnol 28(1):57–82
Venugopal V (2008) Marine products for healthcare: functional and bioactive nutraceutical compounds from the ocean. CRC Press
Vikøren LA, Nygård OK, Lied E, Rostrup E, Gudbrandsen OA (2013) A randomised study on the effects of fish protein supplement on glucose tolerance, lipids and body composition in overweight adults. Br J Nutr 109(4):648–657
Vildmyren I, Cao HJV, Haug LB, Valand IU, Eng Ø, Oterhals Å et al (2018) Daily intake of protein from cod residual material lowers serum concentrations of nonesterified fatty acids in overweight healthy adults: a randomized double-blind pilot study. Mar Drugs 16(6):197
Villamil O, Váquiro H, Solanilla JF (2017) Fish viscera protein hydrolysates: production, potential applications and functional and bioactive properties. Food Chem 224:160–171
Wang K, Siddanakoppalu PN, Ahmed I, Pavase TR, Lin H, Li Z (2020) Purification and identification of anti-allergic peptide from Atlantic Salmon (*Salmo salar*) byproduct enzymatic hydrolysates. J Funct Foods 72:104084
Wang K, Li Y, Zhang Y, Luo X, Sun J (2022) Improving myofibrillar proteins solubility and thermostability in low-ionic strength solution: a review. Meat Sci 189:108822
Windsor M, Barlow S (1981) Introduction to fishery by-products (pp xv+-187pp)
Wu D, Zhang S, Sun N, Zhu B, Lin S (2020) Neuroprotective function of a novel hexapeptide QMDDQ from shrimp via activation of the PKA/CREB/BNDF signaling pathway and its structure–activity relationship. J Agric Food Chem 68(24):6759–6769
Xu N, Peng XL, Li HR, Liu JX, Cheng JSY, Qi XY et al (2021) Marine-derived collagen as biomaterials for human health. Front Nutr 8:702108
Yanat M, Schroën K (2021) Preparation methods and applications of chitosan nanoparticles; with an outlook toward reinforcement of biodegradable packaging. React Funct Polym 161:104849
Yang SC, Baldwin RE (2017) Functional properties of eggs in foods. In: Egg science and technology. CRC Press, Boca Raton
Yang J, Ching YC, Chuah CH, Liou NS (2020) Preparation and characterization of starch/empty fruit bunch-based bioplastic composites reinforced with epoxidized oils. Polymers 13(1):94
Yu C, Wu F, Cha Y, Zou H, Bao J, Xu R, Du M (2018) Effects of high-pressure homogenization on functional properties and structure of mussel (*Mytilus edulis*) myofibrillar proteins. Int J Biol Macromol 118:741–746
Yusmaniar Y, Syafei DI, Arum M, Handoko E, Kurniawan C, Asali MR (2019) Preparation and characterization of seaweed based bioplastic blended with polysaccharides derived from various seeds of avocado, jackfruit and durian. J Phys 1402(5):055097
Zamora-Sillero J, Gharsallaoui A, Prentice C (2018) Peptides from fish by-product protein hydrolysates and its functional properties: an overview. Mar Biotechnol 20:118–130
Zhou X, Chen H, Lyu F, Lin H, Zhang Q, Ding Y (2019) Physicochemical properties and microstructure of fish myofibrillar protein-lipid composite gels: effects of fat type and concentration. Food Hydrocoll 90:433–442

Pharmaceutical and Hydrogel Applications of Marine Collagen

7

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Abstract

The collagen derived from marine sources such as scales and bones of fishes has achieved focus in pharmaceutical industry. Its recent innovation as a biocompatible hydrogel raw material has also enhanced the interest among the researchers in the particular fields due to their specific characteristics. The pharmaceutical industry utilizes marine collagen as a drug delivery tool, with the capability to promote controlled drug release and excellent bioavailability properties. The biocompatibility and lower risk of immunogenicity qualify marine collagen as an ideal choice for healing of wounds, engineering tissue including regenerative medicinal functions. In the application of marine collagen as a hydrogel, it exhibits crucial characteristics by assisting in establishing a biocompatible matrix that simulate the extracellular conditions. Such hydrogels are applied in drug delivery systems with the capacity of sustained release of therapeutic drugs. Furthermore, the innate bioactivity of marine collagen advocates cell connection and propagation, which are fundamental for tissue repair. The versatile nature, paired with its availability in the marine environment, emphasizes its prospective for enhancing pharmaceutical preparations and hydrogel-based technologies, aiding to develop novel solutions in the fields of biotechnology and medicine. This chapter discusses the pharmaceutical and hydrogel applications of collagen isolated from marine resources.

Keywords

Collagen · Hydrogel · Drug delivery · Antiaging · Antioxidant · Antihypertensive

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7.1 Introduction

Collagen derived from marine sources has gained considerable attention as a promising biomaterial across diverse fields. Its abundance, biocompatibility, biodegradability, and similarity to components found in the body's extracellular matrix make it an ideal scaffold in tissue engineering. However, marine collagen, while abundant, typically exhibits lower crosslinking and mechanical strength compared to its bovine counterpart (Coppola et al. 2020). Enhancing its mechanical properties often involves crosslinking treatments. In tissue engineering for bone regeneration, marine collagen scaffolds are often fortified with bioceramics resembling natural bone components, improving mechanical strength, osteoconductive ability, and cell attachment surfaces (Rico-Llanos et al. 2021). Beyond tissue engineering, marine collagen finds applications in dentistry, serving as membranes, bone graft materials, local drug delivery agents, and hemostatic agents. Collagen membranes aid in periodontic and implantation therapies, guiding soft tissue revival and controlling prompt skin re-growth during bone implantation (Sbricoli et al. 2020). These membranes, mostly resorbable within a few days, are commonly used for drug delivery, incorporating substances like chlorhexidine or tetracycline for controlled drug release as collagen degrades (Coppola et al. 2020). Marine collagen also contributes to controlling bleeding, available in sponge-like structures with high absorbency, forming artificial clots to stop bleeding and resorbing within 14–56 days (Wang et al. 2022). The hydrophilic nature and molecular structure of collagen promote cell bonding and damage repair in dental medicine and surgical treatment. Collagen's surface geometry suits cell adhesion, while fibrogenic cells' adhesion is facilitated by fibronectin on the collagen surface (Formentín et al. 2018). Collagen wound dressings come in sponge or film forms, with sponge porosity and fibrous structure influencing cellular growth. Collagen films, produced by casting and crosslinking, offer improved handling properties via UV radiation (Stylianou et al. 2014). In drug delivery, marine collagen's utilization involves studying aspects like stability, bioavailability, solubility, tissue absorption, and target-specific delivery. Nanotechnologies aid in developing new drug delivery systems, leveraging collagen's properties. Marine collagen's abilities extend to wound healing and skin repair, making it a carrier for L-cysteine hydrochloride in topical formulations, supporting wound healing processes (Geahchan et al. 2022). Cosmeceutical commodities which contain aquatic collagen have shown effects comparable to mammal collagen with respect to skin pH, water content, and regulation of sebum. Aquatic collagen hydrolysate exhibits an inhibitory influence on photo-induced aging and offers potential in penetrating the skin due to its small molecular weight (Coppola et al. 2020). The focus on collagen extracted from marine sources highlights its diverse applications and potential benefits across various industries.

7.2 Pharmaceutical Applications of Marine Collagen

Effective utilization of food side streams generated by the industries has been the focal point of the research in the recent times. The demand for byproducts from the food industry has stimulated attempts to transform these side streams into useful resources. Among these different byproducts derived from food side streams, extraction of collagen and its derivative gelatin has gained particular attention as a significant opportunity resultant of their diverse applications in pharmaceutical and biomedical industries. Collagen and its derivative gelatin were principally extracted historically from terrestrial animals like pigs and cows. Conversely, their extraction from non-conventional sources, particularly from marine sources, has gained momentum in current society. Certain religious tabus and concerns regarding certain disease transmissions have led to this scenario. Different studies have explored extraction methods and functional properties of collagen and gelatin from marine sources. This compilation aims to consolidate information on the biomedical and pharmaceutical applications of collagen and gelatin derived specifically from waste generated during fish processing. Figure 7.1 indicates the pharmaceutical application options for the marine collagen.

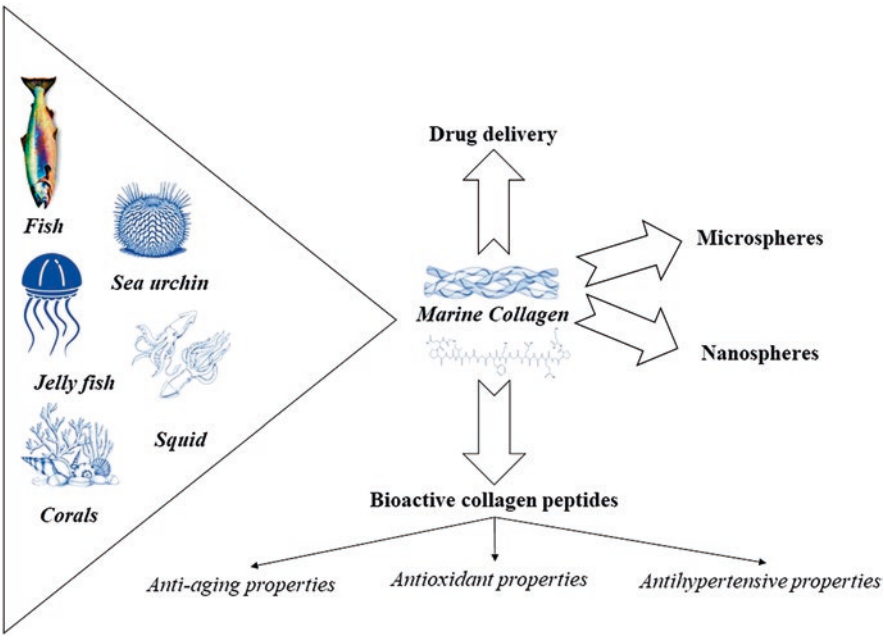

Fig. 7.1 Pharmaceutical application options for marine collagen

7.2.1 Drug Delivery

Natural polymers like collagen and gelatin have gained prominence in drug delivery systems due to their remarkable biocompatibility compared to synthetic polymers. Collagen, specifically collagen type I, possesses a triple-helical peptide structure that can self-accumulate into fibrous form and is biodegradable when subjected to collagenases (Amirrah et al. 2022). Studies have extensively explored various formats of these proteins, such as protein films, gels, microspheres, and nanospheres, for drug delivery applications. However, using big-sized materials in drug transport presents several roadblocks, comprising in-vivo instability, meager bioavailability, solubility matters, incompetent body tissue absorption, achieving objective-explicit delivery, ensuring stimulant efficiency, and potential adversative properties (Patra et al. 2018). Despite these challenges, the exceptional biocompatibility of natural polymers like collagen and gelatin remains a driving force for their utilization in controlled protein delivery systems. Their biodegradability, along with their ability to form various structural formats, makes them promising candidates for drug delivery applications, offering potential solutions to some of these challenges. The development of novel drug delivery systems targeting specific body parts has become crucial in addressing various challenges encountered in conventional drug delivery (Zhang et al. 2021). Nanotechnology has emerged as a pivotal tool in crafting advanced drug formulations, ensuring controlled drug release and delivery, leading to significant success in modern medicine. Numerous experiments have highlighted the potential of collagen as a transferor in these innovative drug transfer methods. For instance, in the realm of skin tissue engineering, researchers designed scaffold-controlled release systems utilizing silver carp skin collagen, chitosan, and chondroitin sulfate scaffolds (Cao et al. 2015). These scaffolds, incorporated with bFGF-loaded PLGA microspheres, displayed controllable protein delivery rates. Subject to the ratio of collagen toward chitosan, these scaffolds exhibited favorable biocompatibility, promoted fibroblast cell proliferation, and facilitated skin tissue regeneration. This proposes the capability of these collagen-based loaded scaffolds for wound therapeutic and skin tissue engineering applications. In another study, researchers developed core-shell marine collagen peptides chelated calcium/calcium alginate nanoparticles with marine collagen peptides sourced from scales of Synodontidae fishes and calcium alginate encapsulation for calcium (Lim et al. 2019). In-vivo experiments indicated enhanced calcium engagement, prevention of calcium deficit, and augmented density of femur bone mineral and calcium content in rats, suggesting that nanoparticles from aquatic collagen peptides-based could serve as an effective transporter for calcium subjunction. Moreover, collagen films and gels created utilizing ASC and PSC from skin of eel fish were evaluated for transferring antibiotics such as tetracycline and ampicillin (Chen et al. 2019). These collagen-based gels and films demonstrated efficient carrier properties for antibacterial and antifungal drugs in drug delivery systems, showcasing their potential application in targeted drug delivery. These studies underscore the versatility and promise of collagen-based materials in designing efficient and targeted drug delivery systems across various therapeutic domains. Utilizing atelocollagens from chum

salmons, a composite gel comprising chitosan and marine collagen was injected subcutaneously in rats (Lim et al. 2019). This gel effectively controlled inflammatory cell subversion and tumor-necrosis factor-α (TNF-α) issue. Moreover, it facilitated the replacement of injected gel with fibrous tissue composed of fibroblasts and extracellular matrix (ECM), suggesting its potential as a transporter for tissue-filler and drug transport methods. Natural marine sponge skeletons were employed to develop new bio-based formulations for absorbing wound exudate (Langasco et al. 2017). The collagen-skeleton of marine sponges functioned as a biocompatible transporter for cargoing L-cysteine hydrochloride, which is a sulfur amino acid acknowledged for its wound healing characteristics. This scaffold's glycosaminoglycans facilitated wound healing processes, positioning it as a biomimetic carrier for wound management. A novel delayed-release tablet coating was formulated using collagen sourced from the marine sponge *Chondrosia reniformis* (Nicklas et al. 2009). These coated tablets exhibited resistance to exposure to hydrochloric acid (HCl). While disintegration appeared in a phosphate buffer solution of pH 6.8, the coating demonstrated appropriateness for prolonged release tablets, exhibiting appropriate mechanical properties and storage solidity. These innovative applications underscore the versatility and promising roles of marine sponge-derived collagen across various drug delivery systems and medical interventions.

7.2.2 Microspheres and Nanospheres

Gelatin microspheres have been employed as carriers for delivering cancer drugs like methotrexate through parenteral routes. Interestingly, when comparing gelatin microspheres crosslinked with 20% glutaraldehyde to non-crosslinked microspheres, there was no discernible difference in the release rate of apomorphine, indicating that the crosslinking process didn't significantly alter the drug release rate (Narayani and Panduranga Rao 1996). Highly crosslinked collagen-gelatin microspheres have demonstrated potential in sustaining the release of highly hydrophilic drugs (Dong et al. 2021). These microspheres exhibited kinetics of the zero-order in the delivery profiles of the drugs they encapsulated. This sustained release pattern is attributed to their minor dimension, big surface field, superior adsorbent competence, and capability to dissolve in water, forming a flawless colloidal mixture. These features make them promising for controlled and sustained drug delivery systems. Microspheres based on collagen have been used to uphold the delivery of IL-2, an immune system signaling molecule (Li et al. 2021). Collagen which is crosslinked has been employed to slow down the delivery of insulin, showing promise for controlled insulin delivery. Additionally, collagen hydrogels have been utilized as carriers for cisplatin, a chemotherapy drug used in cancer treatment (Ma et al. 2022). In wound healing applications, collagen-heparin hydrogels have been effective delivery vehicles. Collagen gels loaded with liposomes have demonstrated the ability to sustain the release of insulin and growth hormone for an extended period, surpassing a week. This sustained release capability can be advantageous in long-term drug delivery scenarios (Wang et al. 2023). Chemically altered collagen,

such as succinylated collagen, has been explored for the controlled in-vivo release of drugs like gentamicin and pilocarpine (Sripriya et al. 2004). Notably, the issue of drugs into the total flow was observed to be lengthier from collagen gel-embedded liposomes compared to the release from vesicles only. Molecules of collagen appear to reduce permeability of liposome through both an antioxidant result and an individual interface with phospholipids, potentially offering a means of fine-tuning drug release kinetics (Pajean and Herbage 1993). The surface area and adsorptive capacity of microspheres significantly influence the drug release profile (Lin et al. 2018). For instance, modifying gelatin microspheres through emulsion crosslinking can alter the release kinetics. In one study, gelatin (type B) and fish gelatin microspheres packed with propranolol-HCl were created using glutaraldehyde as a coupling instrument (Al-Nimry et al. 2021). The formulation demonstrated an impressive entrapment efficiency of 92.38 ± 0.97% and persistent drug delivery for above 11 h, showing promise for controlled drug delivery. Another investigation involved microspheres loaded with fish gelatin ciprofloxacin, a weakly water-soluble antibacterial instrument, formulated through spray-drying (Silva et al. 2018). The particles' average diameter ranged from 2 to 3 μm, with a loading effectiveness exceeding 94%. The medicine was circulated within the initial 6 h. Notably, the ciprofloxacin-loaded microspheres exhibited antibacterial activity against *E. coli* and *Staphylococcus aureus*, displaying minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) estimates similar to absolute ciprofloxacin. The method of spray-drying presented advantages over emulsions, offering higher productivity and potential applicability in pulmonary drug delivery. Collagen and gelatin nanoparticles are formed through a combination of electrostatic and electropic forces, often employing sodium sulfate as a dissolving reagent to enhance interactions between plasmid DNA and collagen (Khan and Khan 2013). The stability of these nanoparticles is significantly influenced by the molecular weight of collagen or gelatin. Factors like pH and temperature affect the molecular weight profile of collagen solutions, influencing the noncovalent interactions responsible for their structure. Studies have assessed the polyion complexation between basic fibroblast growth factor and gelatin, revealing that electrostatic interactions predominantly drive the complexation between acidic gelatin and basic fibroblast growth factor (Muniruzzaman et al. 1998). These biodegradable collagen-based nanoparticles or nanospheres demonstrate thermal stability, enabling effective sterilization. Additionally, they can be taken up by the reticuloendothelial system, enhancing the uptake of compounds, such as anti-HIV drugs, particularly in cells like macrophages (Khan and Khan 2013). As a result, collagen-based nanoparticles have been used as carriers for parenteral delivery of cytotoxic agents and therapeutic compounds like camptothecin and hydrocortisone (Khan and Khan 2013). Their potential extends to sustained release formulations for antimicrobial agents or steroids. Collagen nanoparticles have shown promise in enhancing dermal delivery of substances like retinol, demonstrating higher stability and faster transportation through the skin compared to freshly precipitated drugs (Arun et al. 2021). These attributes highlight the potential of collagen-based nanoparticles as versatile carriers in various drug delivery applications.

7.3 Pharmaceutical Properties of Marine Collagen Peptides

In recent times, there has been a notable focus on peptides derived from the food protein enzymatic breakdown. Such bioactive peptides are gaining attention as potential non-pharmacological alternatives for promoting health and beauty, as well as preventing and managing conditions related to collagen deficiency or dysfunction. Numerous studies indicate that low-molecular weight peptides of collagen, predominantly those containing residues of C-terminal Pro or Hyp, demonstrate various bioactivities, comprising immunomodulation, antibacterial effects, antioxidative properties, and ACE inhibition (Kuprina et al. 2023). Researchers have explored the in-vivo impact of orally administered collagen peptides, revealing their susceptibility to gastrointestinal digestion (Larder et al. 2021). While some peptides exhibit in-vitro activity, they may lose their efficacy after undergoing hydrolysis by peptidases in the gastrointestinal tract. Notably, peptides with C-terminal Pro or Hyp residues could traverse the intestinal wall, flow into circulation in substantial volumes, and manifest bioactivity (Schunck et al. 2015). To exert their intended biological functions, bioactive peptides must be intactly absorbed from the beginning of intestine and resist mortification by plasma-peptidases to arrive at target locations in a functioning form. Under optimal circumstances, collagen, whether in its denaturized form of gelatin or un-denatured form, can be transformed into hydrolysates of collagen or gelatin. Enzymolysis, a commonly employed hydrolysis method, involves treating collagen with proteases like flavourzyme, pepsin, papain, properase E, and trypsin to generate small bioactive peptide chains (Felician et al. 2018).

7.3.1 Antiaging Properties

The quality and health of the skin are intricately tied to the proper structure and functioning of its components. Sustaining healthy skin involves supplementing and safeguarding these structures and functions. Various factors, including UV radiation, hormones, nutrition, and the aging process, can significantly influence the appearance, structure, and integrity of the skin (Addor 2018). Aging, especially chronologic or intrinsic aging, leads to the degradation of the skin's extracellular matrix, resulting in changes such as diminished elasticity, increased wrinkles, and reduced epidermal thickness (Zhang and Duan 2018). Skin aging is not solely a consequence of chronological age; external and environmental factors also play a role, causing qualitative and quantitative alterations like irregular dryness, pigmentation changes, sallowness, skin atrophy, and reduced collagen content. The decline in collagen type I is particularly associated with aging skin (Varani et al. 2006). Consuming collagen peptides orally has shown promise in providing various benefits to the body. For instance, collagen peptide supplements can potentially enhance minimal bone mineral intensity in malnourished individuals and those with deteriorating joint disorders (König et al. 2018). Reports suggest positive effects on hair thickness, nail disorders, collagen fibril size in tendons, fibroblast density, and

collagen fibril formation in the dermis (Wang 2021). Particular collagen peptides within a molecular weight range from 2 to 6 kDa have demonstrated the ability to mitigate skin damage caused by UV radiation and enhance moisture absorption (Li et al. 2022). Preclinical studies indicate that collagen peptides can counteract the impact of UV radiation on mouse skin, improving moisture retention and alleviating damage. The mechanisms of action involve immune enhancement, moisture and lipid retention, antioxidative properties, inhibition of glycosaminoglycan increase, repair of endogenic collagen and fibers of elastin, and sustaining the proportion of collagen type III to type I (Li et al. 2022). Ongoing research is exploring the oral consumption of collagen hydrolysate for enhancing age-associated conditions of skin, especially in aged individuals or due to ecological aspects like photoaging and nourishment. Clinical studies have demonstrated that the oral intake of collagen hydrolysate over several weeks can result in improved elasticity of skin, moistness, and consistency with minimal or no side consequences (Pu et al. 2023). Products like celergen, which contains marine collagen peptides from deep-sea fish sources combined with plant-derived antioxidants, have been clinically confirmed to be valuable and safe for promoting skin advantage (Pérez-Sánchez et al. 2018). Nutricosmetics, or orally consumed nutritional supplements, are increasingly recognized for their role in contributing to healthy skin, complementing the impact of topical creams and cosmetic products that affect the skin externally.

7.3.2 Antioxidant Properties

Biological oxidation is a fundamental activity in aerobic organisms, encompassing animals, plants, and many microorganisms, primarily serving to generate functional-energy. Conversely, this essential activity can result in the development of reactive oxygen species (ROS) and reactive nitrogen species (RNS) as byproducts, including free radicals and non-free radical species (Warraich et al. 2020). The oxidation primarily targets unsaturated fatty acids through a mediated process by free radicals, leading to the formation of lipid peroxyl radicals (Ayala et al. 2014). These radicals perpetuate a chain reaction, initiating destruction to essential organic particles such as proteins, lipids, DNA, and enzymes through peroxidation and nitration processes. Elevated levels of free radicals in the body have been related with numerous pathophysiological settings, including heart ailment, arthritis, cancer, diabetes, lung disease, neurodegenerative diseases (Parkinson's and Alzheimer's), auto-immune diseases, and eye conditions such as macular de-generation (Checa and Aran 2020). Even though the body employs endogenic free-radical foraging antioxidants like ascorbic acid and α-tocopherol, and supplementary natural defensive processes against oxidation, these mechanisms may not be sufficient to prevent ailments associated with free radicals. Antioxidants, such as ascorbic acid, tocopherols, and flavonoids, are recognized to enhance the antioxidant defense of the body (Ali et al. 2020), but ongoing research aims to discover more. Numerous marine collagens with antioxidant activity have been identified from sources such as salmon fish skin, squid skin, jellyfish gonads, jellyfish umbrella, skin of whale shark, skin of Spanish

mackerel, and proteins from tuna backbone (Felician et al. 2018). While there are claims of antioxidant activity in marine collagen-derived peptides, the precise mechanisms through which these peptides exert their effects are not fully understood. Their antioxidant activity is often attributed to their capability in free-radical scavenging, chelate and reduce potential, and safeguard DNA from hydroxyl radical-induced destruction (Wang et al. 2013). The incidence of elevated concentration pf Proline and Glycine in their amino acid sequences contributes to their radical foraging endeavor by contributing protons to electron-lacking radicals (Karami and Akbari-Adergani 2019). Additionally, molecular weight plays a significant role, with lower molecular weight peptides exhibiting higher radical scavenging ability (Jha et al. 2014) and larger molecular weight fractions displaying greater emulsifying properties (Cao et al. 2021), making them potential antioxidants for the food industry. Peptides from salmon fish, for instance, may serve as reduction agents and free-radical scavengers opposing food oxidation, along with antioxidant properties protecting cells from oxidation-induced DNA damage (Wu et al. 2017).

7.3.3 Antihypertensive Properties

Cardiovascular diseases stand as the primary causes of global mortality annually, with hypertension emerging as a prevalent cardiovascular condition contributing to masses of casualties in advanced and developed nations. The renin-angiotensin system (RAS), or renin-angiotensin-aldosterone system (RAAS), plays a pivotal function in controlling artery blood pressure (Muñoz-Durango et al. 2016). Within this system, angiotensin-converting enzyme (ACE), a nonspecific dipeptidyl carboxypeptidase, holds significance. ACE elevates blood pressure via catalyzing the transformation of dormant angiotensin-I into angiotensin-II, a powerful vaso-constrictor, while likewise deactivating bradykinin, an effective vasodilator (Ahmad et al. 2023). The resultant contraction of arterioles leads to enhanced arterial blood pressure. Angiotensin-II additionally promotes retention of sodium and fluid, enhances benevolent adrenergic utility, and induces cardiac and vascular remodeling (Miller and Arnold 2019). Discretion of ACE is a recognized strategy to decrease blood pressure, as it diminishes the Angiotensin-II production. Synthetic ACE inhibitors like captopril, lisinopril, and enalapril have proven effective in hypertension treatment (Messerli et al. 2018). However, their use is related with various side effects, including skin irritation, dry cough, headaches, and angioedema. Antihypertensive peptides derived from food protein offer a potentially safer alternate to synthetic antihypertensive drugs. Across the last 20 years, natural sources of angiotensin-converting enzyme inhibitory peptides (ACEIPs) have been explored, including milk, soybean casein, edible mushrooms, whey, and wheat (Daskaya-Dikmen et al. 2017). Collagen, an intriguing source of ACEIPs, has gained attention for its potential clinical use as a moderate antihypertensive medicine (Ichimura et al. 2009). The exploration of these natural sources aims to provide effective alternatives with fewer side effects compared to synthetic drugs, offering potential benefits in the management of hypertension. The relationship of structure activity of ACE-inhibitory

peptides derived from food is not yet completely understood, but several studies suggest that the fusing of peptides of collagen to ACE is sturdily affected by the peptide's C-terminal tripeptide sequence (Alemán et al. 2011). ACE tends to favor substrata or competitive inhibitors encompassing hydrophobic amino acid remains at each of the 3 C-terminal points. The most potent ACE-inhibitor peptides that often feature a C-terminal tripeptide sequence with collagen peptides (Hyp-Gly-Pro) obtained from jellyfish (*R. esculentum*) have demonstrated a significant reduction in blood pressure in cases of renal vascular hypertension (Zhuang et al. 2012). The amino acid analysis of these peptides revealed elevated levels of Gly, Pro, and Ala, which play crucial roles in ACE-inhibitory and antioxidant activities. The presence of hydrophobic residues in the peptide sequence may also contribute to the ACE-inhibitory and antioxidant activities of collagen peptides (Barzideh et al. 2014). It's worth noting that ACE-inhibitory peptides may also exhibit antioxidant activities in vitro. Collagen peptides with dual ACE-inhibitory and antioxidant activities have been identified in hydrolysates of jellyfish, squid skin, Pacific cod skin, Nile tilapia, cartilage, and chum salmon fish skin (Barzideh et al. 2014; Alemán et al. 2013; Himaya et al. 2012; Chen et al. 2021; Xu et al. 2023). Many scientists suggest that marine collagen peptides derived from these sources hold potential applications as food additives, as ingredients for functional foods, as well as in nutraceutical and pharmaceutical products. The multifunctional nature of these collagen peptides makes them promising candidates for various health-promoting roles.

7.4 Hydrogel-Based Applications of Marine Collagen

According to Morteza et al., (Morteza et al. 2016), hydrogel could be defined as a three-dimensional (3D) setup of hydrophilic polymers that can absorb and preserve a significant volume of water while sustaining structural stability. This unique characteristic arises from the presence of hydrophilic groups linked to the polymeric backbone and the crosslinks between network chains. Hydrogels, with their ability to swell in water, find applications in various fields, and they can be constructed from diverse materials, including both natural and synthetic options (Ho et al. 2022). In contemporary years, there has occurred an increased focus on collagen-extraction from fish and its applications in biomedical engineering. Fish collagen offers advantages in terms of economic production, utilizing discarded byproducts from routine fish processing, and overcoming issues related to biological contaminants and religious considerations associated with mammalian sources (Rajabimashhadi et al. 2023). Fish collagen finds use in the food industry, cosmetics, health care products, pharmaceuticals, and various industrial applications. Fish gelatin, in particular, stands out for its economic viability, as it utilizes byproducts from routine fish processing, making it cost-effective compared to mammalian gelatin. Fish gelatin exhibits superior solubility in water, and understanding the gelation process is crucial. Microvascular endothelial cell hydrogels from customized fish collagen peptide have gained consideration in biomedical applications due to their non-toxic nature, effective water solubility, biocompatibility, and tenable properties

(Zulkifli et al. 2022). The properties of collagen peptide hydrogels can be further enhanced by modifying the monomer and crosslinker, and various methods, including UV or heat curing, can be employed for radical polymerization. To optimize hydrogel properties, fillers can be added, enhancing characteristics such as mechanical properties, degradation behavior, and cell-material interactions (Utech and Boccaccini 2016). Incorporating synthetic materials into networks of polymeric hydrogels through combining collagen with other polymers is proposed as a replacement strategy to enhance the overall performance of the hydrogel. This versatile approach expands the potential applications of hydrogels in diverse fields.

The natural process of cartilage self-repair faces challenges, primarily linked to constrained vascularization of tissue, resulting in a restricted replicative latent of chondrocytes and diminished propensity for migration into damaged sites (Tuan et al. 2013). Despite these challenges, chondrocytes are recognized for their versatility, possessing renewal and differentiation capabilities, making them suitable for various applications. Regenerative medicine approaches, specifically autologous chondrocyte transplantations through tissue engineering, show promise in regeneration strategies which are cell-based, leading to the restoration of impaired cartilage (Makris et al. 2015). Adult nasal chondrocytes are particularly noteworthy as a result of their exclusive traits, originating from the neural crest and demonstrating adaptability to heterotopic transplantation sites (Pelttari et al. 2017). Notably, nasal septum chondrocytes exhibit improved duplicability in producing hyaline like cartilage tissues with excellent malleability, making them adept at adapting to joint environments, ultimately enhancing tissue regeneration and repair (Mumme et al. 2016). However, in-vitro separation and extension of nasal chondrocytes are essential for obtaining suitable cell amount, and protecting their separated state remains a major challenge during in-vitro culturing (Vedicherla and Buckley 2017). Efforts are made to prevent dedifferentiation, often induced by reduced collagen type II production and increased collagen type I deposition (Lefebvre et al. 1990). Strategies include the use of evolution and discrimination factors like TGF-β and the growth of 3D scaffolds mimicking the physical conditions, offering mechanistic stimulus (Nikolova and Chavali 2019). While various synthetic polymers are employed to create 3D scaffolds, they may require structural modifications or surface functionalization to enhance biocompatibility. Combining collagen with other bioactive molecules is a common practice to modify these polymers. Hydrogels with highly hydrated 3D networks that mimic the extracellular matrix are favored due to their resemblance to native cartilage (Tsanaktsidou et al. 2022). Organic bio-materials like Matrigel, agarose, and alginate are frequently employed for systems in 3D scaffolds. The amalgamation of organic and artificial polymers provides significant mechanistic solidity and improved biocompatibility, leveraging the collaborative properties of both substances. Notably, injectable hydrogels, formed in situ, offer an intriguing strategy for tissue engineering and cell therapy (Dimatteo et al. 2018). This technology allows for encapsulation of cells and biomolecules, facilitating administration with minimal toxic effects. Injectable hydrogels can also serve as drug release systems, replacing synthetic polymers. Collagen, owing to its exceptional biocompatibility, safety, biodegradability, and fragile antigenicity, is a

preferred resource in biotechnological uses. It is broadly used in tissue engineering, cosmetics, and pharma industries, highlighting its versatile applications in promoting regeneration and therapeutic advancements. Table 7.1 indicates the hydrogel composite application studies from marine collagen.

References

Addor FAS (2018) Beyond photoaging: additional factors involved in the process of skin aging. Clin Cosmet Investig Dermatol 11:437–443. https://doi.org/10.2147/ccid.S177448

Ahmad H, Khan H, Haque S, Ahmad S, Srivastava N, Khan A (2023) Angiotensin-converting enzyme and hypertension: a systemic analysis of various ACE inhibitors, their side effects, and bioactive peptides as a putative therapy for hypertension. J Renin-Angiotensin-Aldosterone Syst 2023:7890188. https://doi.org/10.1155/2023/7890188

Table 7.1 Hydrogel composite applications from marine collagen

Sl. No.	Marine source	Composite component	Experimental conditions	Inference	Reference
1	Soft coral	Alginate	RT-48 h	The hardness and vigour of the hydrogel made of alginate and collagen were higher compared to the alginate hydrogel, exhibiting hyperplastic behaviour similar to human tissues.	Bendtsen and Wei (2015)
2	Fish skin	Chitosan-gelatin	−20 °C for 24 h and lyophilized.	The gel properties are affected by the components, leading to increased swelling in gels with higher chitosan content. Collagen imparts reduced compression and greater flexibility, whereas gelatin results in higher compressibility and lower flexibility.	Tylingo et al. (2016)
3	Fish skin	Pullulan	pH 9.0, RT-90 min and 50 °C for RT-30 min, pH 7.0	A transparent, lucid, and pliable superabsorbent hydrogel with enhanced mechanical properties, suitable for wound healing and biocompatible applications.	Iswarya et al. (2016)
4	Fish skin	Polydopamine	pH 7.4, 4 °C 10 min centrifugation. 37 °C for 4 h to start gelation reaction.	Enhanced thermal resilience and expansion, increased resistance to enzymatic breakdown suitable for biomedical applications.	Zhu et al. (2016)

Alemán A, Giménez B, Pérez-Santin E, Gómez-Guillén MC, Montero P (2011) Contribution of Leu and Hyp residues to antioxidant and ACE-inhibitory activities of peptide sequences isolated from squid gelatin hydrolysate. Food Chem 125(2):334–341. https://doi.org/10.1016/j.foodchem.2010.08.058

Alemán A, Gómez-Guillén MC, Montero P (2013) Identification of ace-inhibitory peptides from squid skin collagen after in vitro gastrointestinal digestion. Food Res Int 54(1):790–795. https://doi.org/10.1016/j.foodres.2013.08.027

Ali SS, Ahsan H, Zia MK, Siddiqui T, Khan FH (2020) Understanding oxidants and antioxidants: classical team with new players. J Food Biochem 44(3):e13145. https://doi.org/10.1111/jfbc.13145

Al-Nimry S, Dayah AA, Hasan I, Daghmash R (2021) Cosmetic, biomedical and pharmaceutical applications of fish gelatin/hydrolysates. Mar Drugs 19(3):145. https://www.mdpi.com/1660-3397/19/3/145

Amirrah IN, Lokanathan Y, Zulkiflee I, Wee MFMR, Motta A, Fauzi MB (2022) A comprehensive review on collagen type I development of biomaterials for tissue engineering: from biosynthesis to bioscaffold. Biomedicines 10(9):2307. https://www.mdpi.com/2227-9059/10/9/2307

Arun A, Malrautu P, Laha A, Luo H, Ramakrishna S (2021) Collagen nanoparticles in drug delivery systems and tissue engineering. Appl Sci 11(23):11369. https://www.mdpi.com/2076-3417/11/23/11369

Ayala A, Muñoz MF, Argüelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Med Cell Longev 2014:360438. https://doi.org/10.1155/2014/360438

Barzideh Z, Latiff AA, Gan CY, Abedin MZ, Alias AK (2014) ACE inhibitory and antioxidant activities of collagen hydrolysates from the ribbon jellyfish (Chrysaora sp.). Food Technol Biotechnol 52(4):495–504. https://doi.org/10.17113/ftb.52.04.14.3641

Bendtsen ST, Wei M (2015) Synthesis and characterization of a novel injectable alginate–collagen–hydroxyapatite hydrogel for bone tissue regeneration. J Mater Chem B 3(15):3081–3090. https://doi.org/10.1039/C5TB00072F

Cao H, Chen M-M, Liu Y, Liu Y-Y, Huang Y-Q, Wang J-H, Chen J-D, Zhang Q-Q (2015) Fish collagen-based scaffold containing PLGA microspheres for controlled growth factor delivery in skin tissue engineering. Colloids Surf B: Biointerfaces 136:1098–1106. https://doi.org/10.1016/j.colsurfb.2015.10.022

Cao W, Shi L, Hao G, Chen J, Weng W (2021) Effect of molecular weight on the emulsion properties of microfluidized gelatin hydrolysates. Food Hydrocoll 111:106267. https://doi.org/10.1016/j.foodhyd.2020.106267

Checa J, Aran JM (2020) Reactive oxygen species: drivers of physiological and pathological processes. J Inflamm Res 13:1057–1073. https://doi.org/10.2147/jir.S275595

Chen J, Gao K, Liu S, Wang S, Elango J, Bao B, Dong J, Liu N, Wu W (2019) Fish collagen surgical compress repairing characteristics on wound healing process in vivo. Mar Drugs 17(1):33

Chen J, Sun S, Li Y, Liu R (2021) Proteolysis of tilapia skin collagen: identification and release behavior of ACE-inhibitory peptides. LWT 139:110502. https://doi.org/10.1016/j.lwt.2020.110502

Coppola D, Oliviero M, Vitale GA, Lauritano C, D'Ambra I, Iannace S, de Pascale D (2020) Marine collagen from alternative and sustainable sources: extraction, processing and applications. Marine Drugs 18(4):214. https://www.mdpi.com/1660-3397/18/4/214

Daskaya-Dikmen C, Yucetepe A, Karbancioglu-Guler F, Daskaya H, Ozcelik B (2017) Angiotensin-I-converting enzyme (ACE)-inhibitory peptides from plants. Nutrients 9(4):316. https://doi.org/10.3390/nu9040316

Dimatteo R, Darling NJ, Segura T (2018) In situ forming injectable hydrogels for drug delivery and wound repair. Adv Drug Deliv Rev 127:167–184. https://doi.org/10.1016/j.addr.2018.03.007

Dong Z, Meng X, Yang W, Zhang J, Sun P, Zhang H, Fang X, Wang D-A, Fan C (2021) Progress of gelatin-based microspheres (GMSs) as delivery vehicles of drug and cell. Mater Sci Eng C 122:111949. https://doi.org/10.1016/j.msec.2021.111949

Felician FF, Xia C, Qi W, Xu H (2018) Collagen from marine biological sources and medical applications. Chem Biodivers 15(5):e1700557. https://doi.org/10.1002/cbdv.201700557

Formentín P, Catalán Ú, Pol L, Fernández-Castillejo S, Solà R, Marsal LF (2018) Collagen and fibronectin surface modification of nanoporous anodic alumina and macroporous silicon for endothelial cell cultures. J Biol Eng 12(1):21. https://doi.org/10.1186/s13036-018-0111-x

Geahchan S, Baharlouei P, Rahman A (2022) Marine collagen: A promising biomaterial for wound healing, skin anti-aging, and bone regeneration. Mar Drugs 20(1):61. https://doi.org/10.3390/md20010061

Himaya SWA, Ngo D-H, Ryu B, Kim S-K (2012) An active peptide purified from gastrointestinal enzyme hydrolysate of Pacific cod skin gelatin attenuates angiotensin-1 converting enzyme (ACE) activity and cellular oxidative stress. Food Chem 132(4):1872–1882. https://doi.org/10.1016/j.foodchem.2011.12.020

Ho T-C, Chang C-C, Chan H-P, Chung T-W, Shu C-W, Chuang K-P, Duh T-H, Yang M-H, Tyan Y-C (2022) Hydrogels: properties and applications in biomedicine. Molecules 27(9):2902. https://www.mdpi.com/1420-3049/27/9/2902

Ichimura T, Yamanaka A, Otsuka T, Yamashita E, Maruyama S (2009) Antihypertensive effect of enzymatic hydrolysate of collagen and Gly-Pro in spontaneously hypertensive rats. Biosci Biotechnol Biochem 73(10):2317–2319. https://doi.org/10.1271/bbb.90197

Ilya Syafiqa Zulkifli N, Ibrahim N, Jaafar M, Teramoto N (2022) The properties of the modified fish collagen peptide hydrogel. Mater Today Proc 66:2738–2741. https://doi.org/10.1016/j.matpr.2022.06.506

Iswarya S, Bhanukeerthi AV, Velswamy P, Uma TS, Perumal PT (2016) Design and development of a piscine collagen blended pullulan hydrogel for skin tissue engineering. RSC Advances 6(63):57863–57871. https://doi.org/10.1039/C6RA03578G

Jha S, Mandal P, Bhattacharyya P, Ghosh A (2014) Free-radical scavenging properties of low molecular weight peptide(s) isolated from S1 cultivar of mulberry leaves and their impact on Bombyx mori (L.) (Bombycidae). J Anim Sci Biotechnol 5(1):16. https://doi.org/10.1186/2049-1891-5-16

Karami Z, Akbari-Adergani B (2019) Bioactive food derived peptides: a review on correlation between structure of bioactive peptides and their functional properties. J Food Sci Technol 56(2):535–547. https://doi.org/10.1007/s13197-018-3549-4

Khan R, Khan MH (2013) Use of collagen as a biomaterial: an update. J Indian Soc Periodontol 17(4):539–542. https://doi.org/10.4103/0972-124x.118333

König D, Oesser S, Scharla S, Zdzieblik D, Gollhofer A (2018) Specific collagen peptides improve bone mineral density and bone markers in postmenopausal women—a randomized controlled study. Nutrients 10(1):97. https://doi.org/10.3390/nu10010097

Kuprina EE, Kiprushkina EI, Abramzon VV, Rogozina EA, Romanenko NY, Mezenova OY, Grimm T, Mörsel T (2023) Obtaining and study of peptide compositions based on hydrolysates of collagen-containing fish raw materials. Fermentation 9(5):458. https://www.mdpi.com/2311-5637/9/5/458

Langasco R, Cadeddu B, Formato M, Lepedda AJ, Cossu M, Giunchedi P, Pronzato R, Rassu G, Manconi R, Gavini E (2017) Natural collagenic skeleton of marine sponges in pharmaceutics: innovative biomaterial for topical drug delivery. Mater Sci Eng C 70:710–720. https://doi.org/10.1016/j.msec.2016.09.041

Larder CE, Iskandar MM, Kubow S (2021) Gastrointestinal digestion model assessment of peptide diversity and microbial fermentation products of collagen hydrolysates. Nutrients 13(8):2720. https://doi.org/10.3390/nu13082720

Lefebvre V, Peeters-Joris C, Vaes G (1990) Production of collagens, collagenase and collagenase inhibitor during the dedifferentiation of articular chondrocytes by serial subcultures. Biochim Biophys Acta 1051(3):266–275. https://doi.org/10.1016/0167-4889(90)90132-w

Li Y, Liu Y, Li R, Bai H, Zhu Z, Zhu L, Zhu C, Che Z, Liu H, Wang J, Huang L (2021) Collagen-based biomaterials for bone tissue engineering. Mater Des 210:110049. https://doi.org/10.1016/j.matdes.2021.110049

Li C, Fu Y, Dai H, Wang Q, Gao R, Zhang Y (2022) Recent progress in preventive effect of collagen peptides on photoaging skin and action mechanism. Food Sci Human Wellness 11(2):218–229. https://doi.org/10.1016/j.fshw.2021.11.003

Lim Y-S, Ok Y-J, Hwang S-Y, Kwak J-Y, Yoon S (2019) Marine collagen as A promising biomaterial for biomedical applications. Mar Drugs 17(8):467

Lin X, Yang H, Su L, Yang Z, Tang X (2018) Effect of size on the in vitro/in vivo drug release and degradation of exenatide-loaded PLGA microspheres. J Drug Deliv Sci Technol 45:346–356. https://doi.org/10.1016/j.jddst.2018.03.024

Ma J, Wang B, Shao H, Zhang S, Chen X, Li F, Liang W (2022) Hydrogels for localized chemotherapy of liver cancer: a possible strategy for improved and safe liver cancer treatment. Drug Deliv 29(1):1457–1476. https://doi.org/10.1080/10717544.2022.2070299

Makris EA, Gomoll AH, Malizos KN, Hu JC, Athanasiou KA (2015) Repair and tissue engineering techniques for articular cartilage. Nat Rev Rheumatol 11(1):21–34. https://doi.org/10.1038/nrrheum.2014.157

Messerli FH, Bangalore S, Bavishi C, Rimoldi SF (2018) Angiotensin-converting enzyme inhibitors in hypertension: to use or not to use? J Am Coll Cardiol 71(13):1474–1482. https://doi.org/10.1016/j.jacc.2018.01.058

Miller AJ, Arnold AC (2019) The renin-angiotensin system in cardiovascular autonomic control: recent developments and clinical implications. Clin Auton Res 29(2):231–243. https://doi.org/10.1007/s10286-018-0572-5

Morteza B, Naimeh M, Mehdi M (2016) An introduction to hydrogels and some recent applications. In: Sutapa Biswas M (ed) Emerging concepts in analysis and applications of hydrogels. IntechOpen. https://doi.org/10.5772/64301

Mumme M, Barbero A, Miot S, Wixmerten A, Feliciano S, Wolf F, Asnaghi AM, Baumhoer D, Bieri O, Kretzschmar M, Pagenstert G, Haug M, Schaefer DJ, Martin I, Jakob M (2016) Nasal chondrocyte-based engineered autologous cartilage tissue for repair of articular cartilage defects: an observational first-in-human trial. Lancet 388(10055):1985–1994. https://doi.org/10.1016/s0140-6736(16)31658-0

Muniruzzaman M, Tabata Y, Ikada Y (1998) Complexation of basic fibroblast growth factor with gelatin. J Biomater Sci Polym Ed 9(5):459–473. https://doi.org/10.1163/156856298X00569

Muñoz-Durango N, Fuentes CA, Castillo AE, González-Gómez LM, Vecchiola A, Fardella CE, Kalergis AM (2016) Role of the renin-angiotensin-aldosterone system beyond blood pressure regulation: molecular and cellular mechanisms involved in end-organ damage during arterial hypertension. Int J Mol Sci 17(7):797. https://doi.org/10.3390/ijms17070797

Narayani R, Panduranga Rao K (1996) Biodegradable microspheres using two different gelatin drug conjugates for the controlled delivery of methotrexate. Int J Pharm 128(1):261–268. https://doi.org/10.1016/0378-5173(95)04323-3

Nicklas M, Schatton W, Heinemann S, Hanke T, Kreuter J (2009) Enteric coating derived from marine sponge collagen. Drug Dev Ind Pharm 35(11):1384–1388. https://doi.org/10.3109/03639040902939239

Nikolova MP, Chavali MS (2019) Recent advances in biomaterials for 3D scaffolds: a review. Bioact Mater 4:271–292. https://doi.org/10.1016/j.bioactmat.2019.10.005

Pajean M, Herbage D (1993) Effect of collagen on liposome permeability. Int J Pharm 91(2):209–216. https://doi.org/10.1016/0378-5173(93)90340-L

Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres M d P, Acosta-Torres LS, Diaz-Torres LA, Grillo R, Swamy MK, Sharma S, Habtemariam S, Shin H-S (2018) Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol 16(1):71. https://doi.org/10.1186/s12951-018-0392-8

Pelttari K, Mumme M, Barbero A, Martin I (2017) Nasal chondrocytes as a neural crest-derived cell source for regenerative medicine. Curr Opin Biotechnol 47:1–6. https://doi.org/10.1016/j.copbio.2017.05.007

Pérez-Sánchez A, Barrajón-Catalán E, Herranz-López M, Micol V (2018) Nutraceuticals for skin care: A comprehensive review of human clinical studies. Nutrients 10(4):403. https://doi.org/10.3390/nu10040403

Pu SY, Huang YL, Pu CM, Kang YN, Hoang KD, Chen KH, Chen C (2023) Effects of oral collagen for skin anti-aging: a systematic review and meta-analysis. Nutrients 15(9):80. https://doi.org/10.3390/nu15092080

Rajabimashhadi Z, Gallo N, Salvatore L, Lionetto F (2023) Collagen derived from fish industry waste: progresses and challenges. Polymers (Basel) 15(3):544. https://doi.org/10.3390/polym15030544

Rico-Llanos GA, Borrego-González S, Moncayo-Donoso M, Becerra J, Visser R (2021) Collagen type I biomaterials as scaffolds for bone tissue engineering. Polymers 13(4):599. https://www.mdpi.com/2073-4360/13/4/599

Sbricoli L, Guazzo R, Annunziata M, Gobbato L, Bressan E, Nastri L (2020) Selection of collagen membranes for bone regeneration: a literature review. Materials 13(3):786. https://www.mdpi.com/1996-1944/13/3/786

Schunck M, Zague V, Oesser S, Proksch E (2015) Dietary supplementation with specific collagen peptides has a body mass index-dependent beneficial effect on cellulite morphology. J Med Food 18(12):1340–1348. https://doi.org/10.1089/jmf.2015.0022

Silva DM, Vyas HKN, Sanderson-Smith ML, Sencadas V (2018) Development and optimization of ciprofloxacin-loaded gelatin microparticles by single-step spray-drying technique. Powder Technol 330:201–209. https://doi.org/10.1016/j.powtec.2018.02.029

Sripriya R, Kumar MS, Sehgal PK (2004) Improved collagen bilayer dressing for the controlled release of drugs. J Biomed Mater Res B Appl Biomater 70(2):389–396. https://doi.org/10.1002/jbm.b.30051

Stylianou A, Yova D, Alexandratou E (2014) Investigation of the influence of UV irradiation on collagen thin films by AFM imaging. Mater Sci Eng C 45:455–468. https://doi.org/10.1016/j.msec.2014.09.006

Tsanaktsidou E, Kammona O, Kiparissides C (2022) Recent developments in hyaluronic acid-based hydrogels for cartilage tissue engineering applications. Polymers (Basel) 14(4):839. https://doi.org/10.3390/polym14040839

Tuan RS, Chen AF, Klatt BA (2013) Cartilage regeneration. J Am Acad Orthop Surg 21(5):303–311. https://doi.org/10.5435/jaaos-21-05-303

Tylingo R, Gorczyca G, Mania S, Szweda P, Milewski S (2016) Preparation and characterization of porous scaffolds from chitosan-collagen-gelatin composite. React Funct Polym 103:131–140. https://doi.org/10.1016/j.reactfunctpolym.2016.04.008

Utech S, Boccaccini AR (2016) A review of hydrogel-based composites for biomedical applications: enhancement of hydrogel properties by addition of rigid inorganic fillers. J Mater Sci 51(1):271–310. https://doi.org/10.1007/s10853-015-9382-5

Varani J, Dame MK, Rittie L, Fligiel SE, Kang S, Fisher GJ, Voorhees JJ (2006) Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. Am J Pathol 168(6):1861–1868. https://doi.org/10.2353/ajpath.2006.051302

Vedicherla S, Buckley CT (2017) Rapid chondrocyte isolation for tissue engineering applications: the effect of enzyme concentration and temporal exposure on the matrix forming capacity of nasal derived chondrocytes. Biomed Res Int 2017:2395138. https://doi.org/10.1155/2017/2395138

Wang H (2021) A review of the effects of collagen treatment in clinical studies. Polymers (Basel) 13(22):3868. https://doi.org/10.3390/polym13223868

Wang B, Wang YM, Chi CF, Luo HY, Deng SG, Ma JY (2013) Isolation and characterization of collagen and antioxidant collagen peptides from scales of croceine croaker (*Pseudosciaena crocea*). Mar Drugs 11(11):4641–4661. https://doi.org/10.3390/md11114641

Wang L, Li W, Qu Y, Wang K, Lv K, He X, Qin S (2022) Preparation of super absorbent and highly active fish collagen sponge and its hemostatic effect in vivo and in vitro [original research]. Front Bioeng Biotechnol 10:862532. https://doi.org/10.3389/fbioe.2022.862532

Wang Y, Chen L, Wang Y, Wang X, Qian D, Yan J, Sun Z, Cui P, Yu L, Wu J, He Z (2023) Marine biomaterials in biomedical nano/micro-systems. J Nanobiotechnol 21(1):408. https://doi.org/10.1186/s12951-023-02112-w

Warraich UE, Hussain F, Kayani HUR (2020) Aging-oxidative stress, antioxidants and computational modeling. Heliyon 6(5):e04107. https://doi.org/10.1016/j.heliyon.2020.e04107

Wu R, Chen L, Liu D, Huang J, Zhang J, Xiao X, Lei M, Chen Y, He H (2017) Preparation of antioxidant peptides from Salmon byproducts with bacterial extracellular proteases. Mar Drugs 15(1):4. https://doi.org/10.3390/md15010004

Xu S, Zhao Y, Song W, Zhang C, Wang Q, Li R, Shen Y, Gong S, Li M, Sun L (2023) Improving the sustainability of processing by-products: extraction and recent biological activities of collagen peptides. Food Secur 12(10):1965. https://doi.org/10.3390/foods12101965

Zhang S, Duan E (2018) Fighting against skin aging: the way from bench to bedside. Cell Transplant 27(5):729–738. https://doi.org/10.1177/0963689717725755

Zhang Y, Davis DA, AboulFotouh K, Wang J, Williams D, Bhambhani A, Zakrewsky M, Maniruzzaman M, Cui Z, Williams RO (2021) Novel formulations and drug delivery systems to administer biological solids. Adv Drug Deliv Rev 172:183–210. https://doi.org/10.1016/j.addr.2021.02.011

Zhu S, Gu Z, Xiong S, An Y, Liu Y, Yin T, You J, Hu Y (2016) Fabrication of a novel bio-inspired collagen–polydopamine hydrogel and insights into the formation mechanism for biomedical applications. RSC Adv 6(70):66180–66190. https://doi.org/10.1039/C6RA12306F

Zhuang Y, Sun L, Li B (2012) Production of the angiotensin-I-converting enzyme (ACE)-inhibitory peptide from hydrolysates of jellyfish (Rhopilema esculentum) collagen. Food Bioprocess Technol 5(5):1622–1629. https://doi.org/10.1007/s11947-010-0439-9

Biomaterials

8

S. Sabu

Abstract

Biomaterials are materials that exhibit a high level of biocompatibility and are designed to interact with biological systems in a variety of ways, such as tissues, organs, cells, and molecules. Biomaterials can be classified into: (i) natural (e.g. collagen, chitosan, dextran, and silk), offer inherent biocompatibility and bioactivity and: (ii) synthetic (e.g. polypropylene, polyurethanes, metals), provide the advantage of precise control over material properties, allowing for customization based on specific applications. For tissue engineering, drug delivery, and other biomedical applications, natural polymers based on proteins and polysaccharides show a lot of promise. Mammalian-derived polymers are preferred over non-mammalian sources because they have a multitude of physiologically active designs and favourable host-material responses. Compared to mammalian collagen, marine collagen (MC), which comes from marine organisms, has excellent physical and chemical robustness, and is readily available in huge amounts. In recent years, tissue engineering and regenerative medicine opt for collagen-based materials over other types of biomaterials on account of their high biocompatibility, low immunogenicity, and structural versatility. This chapter discusses the significance and classification of biomaterials; protein-based and polysaccharide-based biomaterials and the application biomaterials with a special focus on marine-derived collagen.

Keywords

Marine collagen · Chitosan · Tissue engineering · Biomedical applications · Biocompatibility

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8.1 Introduction

Biomaterials are substances with a substantial degree of biocompatibility and are engineered to associate with biological systems for various applications ranging from tissues and organs to cells and molecules. Biomaterials can be of natural or synthetic origin with specific functions to perform in biological environments or human bodies (Smallman and Bishop 1999). The significant activities of biomaterials are augmenting or replacing the functionality of biological tissues and facilitating medical interventions such as implants, prosthetics, drug delivery systems, and tissue engineering. From the pre-historic Egyptians (usage of sutures made from animal sinew) to the modern intelligent wound dressing materials, the significance and the applications of biomaterials have grown significantly due to discoveries in tissue engineering, regenerative medicine, and more. The modern biomaterial field combines biology, chemistry, medicine, physics, and more, representing a fascinating intersection of science, engineering, and medicine, paving the way for innovative advancements in healthcare and technology (NIBIB 2023). Biomaterials can be classified into: (i) natural (e.g. collagen, chitosan, dextran, and silk), offer inherent biocompatibility and bioactivity and: (ii) synthetic (e.g. polypropylene, polyurethanes, metals), provide the advantage of precise control over material properties, allowing for customization based on specific applications (Fig. 8.1). The more homogeneous structures, mechanical strengths, and rates of degradation of synthetic polymers (PLGA: polylactic acid, PGA: polyglycolide, and PLA: polylactic acid) make them easier to produce in large quantities at a low cost. But unlike natural biomaterials, they don't have the same natural cell binding sites, which leads to decreased bioactivities and higher in vivo rejection rates (Troy et al. 2021).

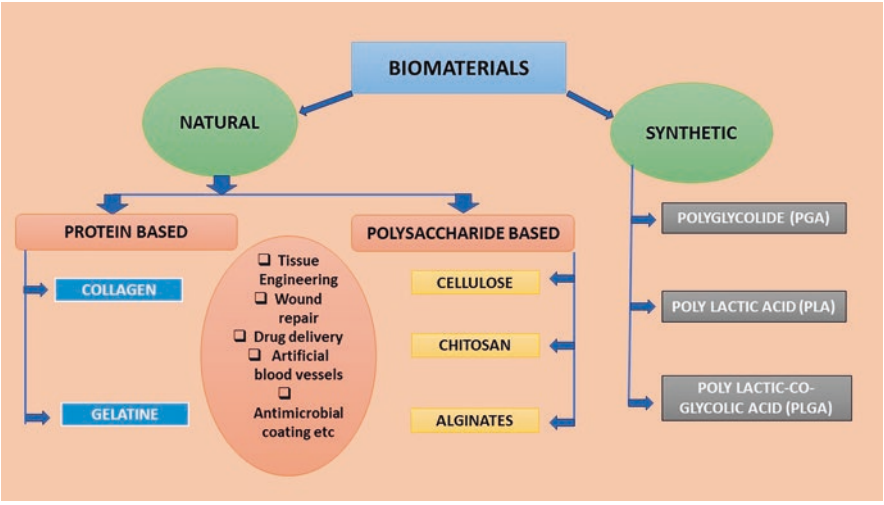

Fig. 8.1 Classification and functions of biomaterials

All biomaterials can intermingle with biological systems both actively or passively, making them the primary contenders for the manufacture of medical devices (Hashmi and Koester 2018). Developing innovative biomaterials for specific applications and advanced research on molecular and cellular interactions between the materials and biological systems through biomedical research is essential to finding solutions to emerging problems in healthcare, such as the ageing population, organ shortages, and the need for more effective therapeutic interventions.

8.2 Natural Biomaterials

Utilizing naturally occurring macromolecules found in plants and animals has become more popular in recent years as a way to advance the biocompatibility and biodegradability of constituents delivered, especially in tissue engineering. Natural biomaterials are well tolerated, encouraging cellular connection, body assimilation, and tissue formation, while their biodegradability allows tissue renovation (Ullah and Chen 2020). One significant advantage of using natural polymers as biomaterials is their mechanical properties. They can be tempered to repel pressure powers, match tissue possessions, and employ mechanical inducement on cells (Bhattarai et al. 2018). Polysaccharides such as cellulose, methylcellulose, starch, alginate, amylose, agarose, chitin, dextran, and glycosaminoglycans are mostly non-mammalian. They are the structural carbohydrates in plants and exoskeletons of crustaceans and arthropods. Proteins, on the other hand, are mainly of mammalian origin (Table 8.1). The materials include collagen, gelatin, fibrin, silk, titin, keratin, mucin, and elastin. Proteoglycans (lumican, versican, aggrecan, and neurocan), lipids, and nucleic acids (DNA, RNA) are the other commonly known materials coming under natural biomaterials and are used as carrier, scaffold, or substrate materials having intrinsic bioactivities (Joyce et al. 2021).

Non-mammalian natural polymers are abundant and readily available. Polymers such as chitosan, alginate, and dextran, derived from plant sources, can be easily extracted and purified, resulting in a cost-effective material with low antigenicity. Drug delivery devices frequently use alginate, a naturally occurring polysaccharide present in seaweed. The basic repeat unit of agarose, which is derived from algae, has 1,3-linked D-galactopyranose and 1,4-linked 3,6-anhydrous α-l-galactopyranose linked to it and is frequently employed as a matrix for cell encapsulation with thermal crosslinking capabilities (Normand et al. 2000). Methylcellulose, derivative of cellulose, endures gelation at 37 °C. In the biomedical industry, cellulose, methylcellulose, and agarose are commonly employed (Joyce et al. 2021).

8.3 Protein-Based Biomaterials

Proteins have been incorporated into protein/polypeptide-based biomaterials in recent years due to their cellular relations, physical chemistry, or cell communication possessions due to the vital structural roles that various proteins play in living

Table 8.1 Methods of isolating biomaterials: properties and biomedical applications

Biomaterial	Method of isolation	Properties	Forms of biomaterial	Applications
Collagen	Physical/ Chemical/ Enzymatic decellularization Solubilization and purification	Low immunogenicity, structural versatility, high biocompatibility	Collagen-scaffolds, hydrogels, sponges, films, and membranes	Regenerative medicine Tissue engineering
Gelatine	Thermal denaturation Hydrolysis of collagen Gelatine type A-soaking in dilute acids Gelatine type B-soaking in alkali	High solubility Biocompatible, biodegradable, cell binding, Lack of antigenicity	Gelatine microparticles	Tissue engineering Drug delivery, food, and nutraceuticals applications
Cellulose	Cellulose-I: native form of cellulose Cellulose-II: alkali treatment of cellulose-I Cellulose-III: ammonia or amide treatment of Cellulose I or II	Stiffness and flexibility, Biocompatible	Nanocellulose Nanofibres, nanocrystals	Drug delivery, wound dressing, artificial blood vessels
Chitosan and chitosan oligosaccharide	Deacetylation of chitin Degradation of chitosan using reducing agents	Biodegradable, Non-toxic, Film forming, Antimicrobial	Nanochitosan, Chitosan gels, Oligomers	Drug delivery, tissue engineering, wound healing, food, antimicrobial films, and membranes etc
Alginate	Aqueous alkali, filtration, and precipitation	Gell formation, Biocompatible	Alginate hydrogels, microparticles, crosslinked alginates, sponges, films	Protein-based drug delivery, wound healing, tissue engineering

organisms. Because they can alter the functional characteristics of biomaterials, non-structural proteins are also drawing interest (Choi et al. 2018).

8.3.1 Collagen Biomaterial

Due to its high biocompatibility, biodegradability, accessibility, and ability to handle large quantities, collagen is extensively utilized as a biomaterial in diverse fields. Collagen is a kind of protein, an exceptional material in drug transfer, tissue engineering, food, cosmetics, and many other fields (Parenteau-Bareil et al. 2010). In recent years, it has aroused a great deal of interest from several researchers due to its large resource base, restricted extraction charges, and good physical and chemical properties. Collagen, constituting around one-third of the body's protein tissue bulk, is a principal protein in soft and hard tissues. It is broadly present in various tissues, including bones, skin, tendons, and teeth. The extracellular matrix comprises 28 identified types of collagens, per Type I collagen being the furthermost widespread, chiefly in tendons and bones (Aszódi et al. 2006). Commercial collagen is mostly derived from pigs or terrestrial mammals, and its extraction has long been a major source of supply for the food, cosmetic, pharmaceutical, and biomedical industries. But, because of occurrences of FMD (foot-and-mouth disease), TSE (transmissible spongiform encephalopathy), and BSE (bovine spongiform encephalopathy) in recent years, there have been increased concerns raised regarding the health effects of using collagen and collagen-derived products from terrestrial animals (Xu et al. 2021). It is mainly made up of the extracellular matrix found in the skin, bones, ligaments, cartilage, and tendons. Type I collagen makes up over 85% of human collagen, with Type II, III, and IV collagen being more prevalent forms (Lim et al. 2019). Collagen molecules consist of three polypeptide chains attached to each other, as in a 3-stranded rope. A great concentration of amino acid and glycine remains as the main factor that affects helix formation. The structure consists of proline, hydroxyproline, and glycine, particular collagen functions are caused by particular amino acids. Hydrogen bonds bind peptide bonds together to form the helix structure (Nair et al. 2010; Khan and Khan 2013).

Compared to mammalian collagen, marine collagen (MC), which comes from marine organisms, has several advantages, has excellent physical and chemical robustness, and is readily available in huge amounts. It is possible to separate marine collagen from marine craniates (marine mammals and fish), algae, and marine invertebrates (sea urchins, octopuses, jellyfish, sea anemones, sponges, squid, cuttlefish, prawns, starfish, etc.). Since marine collagen is water soluble, safe, biocompatible, highly biodegradable, low immunogenic, easy to extract, with high yield, and requires little capital investment during production, it has garnered significant interest from the scientific and industrial communities (Jankangram et al. 2016).

8.3.1.1 Collagen Scaffolds

Collagen scaffolds that are pure or blends of collagen with natural/synthetic polymers can be created from the collagen. The first tissue-engineered meniscal tissue

construct utilized in clinical practice was a collagen scaffold, which is made of extremely porous CMI scaffold composed of Type I collagen fibres. In Europe, this is being offered commercially by Ivy Sports Medicine in Gräfelfing, Germany as a CMI (collagen meniscus implant) (Tarafder and Lee 2016). The performance of collagen scaffolds can be enhanced by blending collagen with natural polymers. Similarly, combining collagen with synthetic polymers in specific engineering applications enables scaffolds to function with ideal mechanical and biological qualities (Dong and Lv 2016). When mixed with collagen, chitosan—a non-immunogenic, biodegradable, positively charged polymer—forms scaffolds with remarkable mechanical and biological attributes and outstanding compatibility with an array of seeded cell types. Blended collagen scaffolds contain synthetic polymers such as polyethylene glycol (PEG) and PLA. In this case, the collagen supplies the binding sites and cell signalling essential for tissue repair, while the synthetic polymer usually enhances the mechanical characteristics and scaffold structure (Zang et al. 2015).

8.3.1.2 Skin Tissue Regeneration

The skin is alienated into two primary sheets: the dermis and the epidermis. Elasticin and collagen (primarily Type I) are produced by fibroblasts found in the latter, while cells make up the former. Due to its mechanical integrity and other properties, collagen defines the physiology of the skin. Dermal matrix assembly and fibroblast migration are regulated by collagen, which in turn controls derived matrix metalloproteinase (MMP)-14 and fibroblast to maintain homeostasis of skin collagen (Ghomi et al. 2021). Since collagen has strong water absorption capabilities, biodegradability, haemostatic, analgesic, and anti-inflammatory effects, and can support cell attachment, treatments for trauma, burns, chronic trauma, infectious or surgical injuries of skin are currently being carried out using it (Cui et al. 2020). For example, Han et al. (2020) created a skin collagen treated with tea polyphenols, which has the advantages of hydrostructural malleability, mechanical cleverness, and shape remembrance for future skin maintenance products. Zheng et al. (2021) employed the collagen sponge scaffold modified with polydopamine as a new template for dermal regeneration, releasing platelet-rich plasma continuously to speed up skin repair.

8.3.2 Gelatine Biomaterials

Gelatine, a famous biodegradable, and biocompatible polymer created when natural collagen breaks and becomes denatured. Collagen is hydrolysed and thermally denatured to create gelatine. It has many medicinal uses and is frequently used as a less expensive collagen substitute in food and cosmetic manufacturing. It is derived from Type I fibrillar collagen and contains pure protein (up to 92%), water, and mineral salts (Bello et al. 2020). An extra hydrolysis procedure using acid or alkali solutions is necessary for non-soluble collagen. Gelatine Type A is produced by soaking in diluted acidic solutions, whereas gelatine Type B is produced by

immersing in alkali solutions. The isoelectric point and amino acid composition of gelatine Type A are more akin to those of collagen (Lee et al. 2016). When collagen is denaturized, a very low-viscosity gelatine solution is created, and at temperatures below 37 °C, gelatine gels form. Due to the lack of tyrosine and tryptophan, as well as the smaller phenylalanine configuration, gelatine is less antigenic than collagen (Troy et al. 2021).

Gelatine's gel-forming properties, along with its biocompatibility and biodegradability, make it the perfect material for microparticle production. Due to their effortlessness of manufacture, steadiness, lack of harmfulness, and capacity to interact with various bioactive compounds, gelatine microparticles are widely used as drug carriers. Smaller particles are employed in vivo to safeguard and regulate the release of bioactive compounds. Enhanced cell attachment and differentiation can be achieved by employing larger microparticles with surface modifications as "microcarriers" of cells (Tielens et al. 2007). Gelatine biomaterial has numerous applications in tissue engineering and drug delivery (Magadala and Amiji 2008; Narayanan et al. 2013).

8.4 Polysaccharide-Based Biomaterials

The macromolecules known as polysaccharides comprise monosaccharide units bonded covalently by o-glycosidic bonds. The occurrence of carboxylic, hydroxyl, and amine functionalities in the polysaccharide structure lead them suitable candidates for structural alterations that could help adjust physicochemical properties through chemical changes. These are acknowledged as eco-friendly materials made from renewable natural resources. As a result, they are used in biomedical applications as green substitutes. Bioavailable sources such as plants, animals, and microorganisms can easily yield these polysaccharides (Sun et al. 2020). Drug delivery and tissue regeneration are two biomedical uses for naturally occurring polysaccharides from the plant and animal kingdoms. These materials facilitate Numerous physiological processes, including the production of antibodies to improve organismal immunity and the development of cytokines that stimulate macrophages and lymphocytes. The human body can, therefore, readily identify and accept biomaterials based on polysaccharides (Tekale et al. 2022).

8.4.1 Cellulose and Nanocellulose

There are several sources of cellulose, including cotton, wood, and certain bacteria. Chemically, cellulose is a carbohydrate comprising units of glucose linked together by β-glycosidic bonds, making a structured fibrillar structure. Due to their environmental friendliness, biomaterials derived from cellulose have a broad application in wound repair, viscosity modifiers, medication delivery, coating materials, and tablet binders among other applications (Sood et al. 2022). The bacterial cellulose generated by aerobic bacteria has various benefits, including exceptional sponginess,

stout water-absorbing ability, high mechanical strength, and good biocompatibility (Sharip and Ariffin 2019). With a free liquid surface electrospinning technique, nano-bioglass (nBG), silk fibroin (SF), and carboxymethyl cellulose (CMC) were electrospun to create nanofibrous composites that emulate the normal bone extracellular matrix. Good physicochemical and biological properties of the developed scaffold allow it to be suitable for hMSCs' osteogenic distinction and growth, making it appropriate substantial for bone tissue applications (Singh and Pramanik 2018). Cotton cellulose is the most freely accessible, lignin-free, and FDA-approved type of cellulose among the many varieties. The scaffold for bone tissue engineering can be made of cotton microfibers (Tekale et al. 2022).

There are currently three types of nanocellulose that have been described; bacterial nanocellulose (BNC), which is produced by bacteria; cellulose nanofibrils (CNF); and cellulose nano crystals (CNC), mutually of which are formed by breaking down pure cellulose (PC) using shear forces and refinery techniques. Utilizing nanocellulose as scaffolds to support cell culture, repair lesions, and regenerate tissue has advanced significantly in recent years (Klemm et al. 2009). It is preferable for celluloses to form nanofiber networks for biomedical applications. This boosts the celluloses' surface area and improves their interactions with polymers and biomaterials (Lin and Dufresne 2014). Wood cellulose nanofibrils (CNFs) of wood have a very high surface area reactivity and are able to replicate the nano-structured collagen that is found in bone extracellular space (Carlström et al. 2020). Because of the mechanical strength and blood biocompatibility of materials based on nanocellulose, there is interest in using bacterial nanocellulose in particular for producing artificial blood vessels. Zang et al. (2015) created a BNC artificial blood vessel with *Gluconacetobacterxylinum* that measured 100 mm in length and 1 mm in thickness. Tetracycline and doxorubicin, which are soluble in water, have been demonstrated to bind to pure CNC produced by acid hydrolysis and release over 24 h (Letchford et al. 2011). At present, there is a wide range of BNC wound healing products on the market. Some of these products, such as Gengiflex (which treats periodontal disease) and BioFil (which treats burns and ulcers), serve two purposes: they hydrate and absorb, maintaining the ideal healing environment (Lin and Dufresne 2014).

8.4.2 Chitosan and Chitosan Oligosaccharides

Chitin, poly (β-(1–4)-N-acetyl-d-glucosamine), the second greatest biopolymer after cellulose, abundant in crustaceans (shrimp, crabs, lobsters), insects, and fungi. Chitin on deacetylation results in chitosan, which is soluble only in an acidic environment (Sabu et al. 2022). Chitosan, derived from chitin through deacetylation, comprises glucosamine and a segment of N-acetyl-glucosamine items. This decomposable and non-toxic polymer exhibits excellent chelating ability, a crucial property for detoxification (Rasweefali et al. 2021). Chitosan is the key fundamental ingredient used to create the more useful polymers for industry, chitosan, and chitosan oligomers (COSs) from chitin. Identical to chitin, chitosan is a copolymer

encompassing GlcN and GlcNAc units, with GlcN predominating. Because of this, chitosan is a well-thought-out deacetylated form of chitin, often determined by the degree of acetylation (DA). Chitooligoosaccharides, also known as chitosan oligosaccharides (COSs), are the byproducts of enzymatic or acid hydrolysis of chitin, chitosan, or both. Smaller chain distances and free amino groups linked to D-glucosamine units characterize COSs. They dissolve in water and have a lower viscosity (Naveed et al. 2019; Sabu et al. 2022). The scientific community became intrigued towards using these composites in pharmaceutical and therapeutic uses, and also for nutraceuticals and cosmeceuticals, due to their attractive physiological effects, non-toxicity, and higher solubility (Swiatkiewicz et al. 2015; Lodhi et al. 2014).

The temperature, length of the extraction process, source, kind of extractants used, and extraction treatment duration all affect the quality of isolated chitin. There is no significant negative impact on the yield and quality of chitin if the order in which the removal of minerals (demineralization) and proteins (deproteinization) is switched (No and Meyers 1995; Rasweefali et al. 2022). Two important quality parameters for chitin and chitosan are their molecular weight (Mw) and degree of deacetylation (DA). Rasweefali et al. (2021) found a noteworthy stimulus of the isolation process on the DD and Mw of chitosan. Determining the potential industrial applications of chitosan requires characterization based on the relationship between structure and property (de Alvarenga et al. 2010). According to Rasweefali et al. (2021), one important factor in ensuring the quality of chitosan is its molecular weight (MW). There are three kinds of chitosan based on Mw: LMWC (low Mw chitosan) (<50 kDa), MMWC (medium Mw chitosan) (50–250 kDa), and HMWC (high Mw chitosan) (>250 kDa). Medium Mw chitosan has anti-cancer properties, while low Mw chitosan has antioxidant, antitumor, and antibacterial qualities (Anraku et al. 2011). Viscosity, deacetylation degree (DA), and polymerization degree (PD) of chitosan are found to be major determinants of its biological characteristics (Basa et al. 2020; Ahmed et al. 2020). Cho et al. (1998) found that chitosan's antimicrobial action rose when viscosity decreased from 1000 to 10 cP.

The reactive amino groups found in the structure are primarily responsible for the applications of chitosan (Shirvan et al. 2019). DD affects biological and physicochemical characteristics, that is, biodegradability, acid-base and electrostatic characteristics, sorption qualities, self-aggregation, and capacity to interact with metal ions (Kumari et al. 2017). The distribution of GlcNAc and GlcN units along the polymeric chain, Mw, DD, solubility, crystallinity, and other factors all have a major effect on the biological characteristics of COS and chitosan (Younes and Rinaudo 2015; Sabu et al. 2022). The different Mws of chitosan and COSs affect their antioxidant activities (Anraku et al. 2011; Inamdar and Mourya 2014: Chang et al. 2018). Anraku et al. (2011) reported significantly higher antioxidant properties for lower Mw chitosan.

Since it can be easily made into 2D films and fibres and 3D scaffolds like hydrogels and sponges, chitosan is used in wound healing (Rodríguez-Vázquez et al. 2015). In wound healing applications, chitosan promotes dermal fibroblast proliferation to expedite re-epithelialization (Howling et al. 2001). Because there was no

cellular toxicity observed, it was discovered that electrospun chitosan/poly (vinyl alcohol) composite nanofiber wound dressings stimulated fibroblast attachment and proliferation (Zhou et al. 2008). When peptide-containing chitosan microspheres are administered via the nose, it has been demonstrated that this makes it easier for the peptides to pass through the nasal barrier, making them stickier and more likely to be absorbed on the nasal surface (Van der Lubben et al. 2003). Wound healing, non-toxicity, and antibacterial qualities are exhibited by chitosan. Applications such as cell ingrowth and osteoconduction benefit from its broad affinity for in vivo macromolecules. The unique characteristics of chitosan-based materials render them a highly promising platform for developing enhanced bio-distribution, heightened specificity, and heightened sensitivity, thereby enabling the production of diverse materials intended for applications in biomedicine (Islam et al. 2017).

8.4.3 Alginates

Alginates, which are hydrophilic and anionic polymers, are among the most extensively distributed biosynthesized materials on the planet. They can be found in the cell walls of bacterial species like Pseudomonas and Azotobacter and brown algae like *Laminaria hyperborea* and *Macrocystis pyrifera.* Because of their extensive availability, affordable price, low toxicity, biodegradability, and strong gelling power, they have received approval from the USFDA for application as a biopolymer in tissue engineering and regenerative medicine (Lee and Mooney 2012; Sun and Tan 2013). Approximately 40% of the dry weight of brown seaweeds can be composed of alginate biopolymers. Usually, aqueous alkali is used to extract alginate, which is then filtered, precipitated, and recovered as a powder that is soluble in water (Rinaudo 2008). Compared to other biomaterials, alginate is mainly used as a hydrogel. The most widely used technique for producing alginate hydrogels is the addition of ionic crosslinking agents, such as divalent Ca2+ cations, which are typically calcium chloride (Lee and Mooney 2012).

For in vivo applications, various methods have been developed to create biodegradable alginates. When alginate partially oxidizes, its gelling properties are not affected, and the alginate backbone conforms to an open chain adduct, increasing its degradability in aqueous environments (Augst et al. 2006). Since mammals do not have cell receptors for alginate polymers, adhesion-promoting coupling of molecules like collagen and fibronectin may be required. It has been more successful in attaching short amino acid chains that promote ECM adhesion than coupling entire molecules, which has proven challenging (Prang et al. 2006; Augst et al. 2006). As a biomaterial, alginate has demonstrated great promise, especially in drug delivery, guided tissue engineering, and wound healing. Other forms of alginate have a low mechanical strength which limits their applications, even though hydrogel shows promising gelling capability, ease of modification, and biocompatibility. It is anticipated that further research will lead to the creation of dressings with significantly higher bioactivities and altered carriers for bioactive and medicinal compounds (Troy et al. 2021).

8.5 Tissue Engineering

Tissue engineering, also known as regenerative medicine, is a hastily rising interdisciplinary arena of life science. It combines biological and engineering principles to generate new tissues and organs and encourage the rejuvenation of diseased or damaged tissues and organs through the combination of body cells and highly porous scaffold biomaterials. Because of its superior biocompatibility, marine collagen has sparked interest in its probable usage in regenerative medicine and tissue engineering to create biomaterial scaffolds (Lim et al. 2019). Considering their outstanding biocompatibility and adaptability, collagen-based biomaterials are highly attractive for replacing and treating human tissues, including tendons, vascular applications, and bones. Collagen, calcium phosphate, and other materials (water, proteins, etc.) make up bones (Coppola et al. 2020). Synthetic bone scaffolds made out of collagen can temporarily substitute actual bones that have been wounded. This provides mechanical integration, cell activity, and activation of osteo-induction and osteointegration with the host tissues (Chen and Liu 2016).

8.5.1 Bone Tissue Engineering

Bone tissue engineering objective is to create advanced replacements for bone tissue, addressing defects and reinstating bone integrity (Li et al. 2021). Natural tissues feature an extracellular matrix (ECM) comprising molecules concealed by cells. These molecules offer spatial and mechanical signs to cells, providing physical backing for tissues. The ECM helps as a scaffold for cell organization in connective tissue and is dynamic in modelling cell performance and tissue function. Therefore, a logical methodology comprises creating a graft that imitates the ECM of the impaired tissue or organ, helping sequential repair. Hydroxyapatite (HA) scaffolds are widely used in tissue engineering, yet HA is fragile and has drawbacks because of bone regrowth and degradation. As an alternative, collagen has been employed because of the intriguing mechanical and biological characteristics of collagen-based scaffolds (Ghomi et al. 2021). Collagen derived from marine sources has numerous uses in bone tissue due to its high hydrophilicity and amino acid content, which creates the ideal extracellular environment. It can increase osteoblast growth and differentiation, and bone marrow mesenchymal stem cells (BMSCs) that stimulate osteoblastic differentiation can still modulate immune responses (Dimitriou et al. 2011). Collagen fibres, precisely Type I collagen, comprise most of the organic matrix found in bones. As a result, Type I collagen—which exists naturally as hydrogel and scaffold—is frequently utilized in bone repair. However, collagen's poor osteogenetic and mechanical strength prevents it from being widely used in bone regeneration. Thus, the addition of some bioceramics that are comparable to the inorganic components of natural bone can significantly improve the mechanical properties, osteogenic/osteoinductive traits, structural stability, porosity, and other characteristics of the collagen matrix (Ghomi et al. 2021; Zhang et al. 2018).

Proliferating osteoblasts were stimulated, and mature osteoclasts were inhibited by MCP that was extracted from the scales of two different species of fish: Chanos and Sparidae. These results suggest that MCP has a high biological activity to stimulate osteoblasts' osteogenic potential and prevent osteoporosis by controlling osteoclast activity. It also promotes bone tissue regeneration (Hu et al. 2016). Tilapia squamous collagen can stimulate osteoblast growth and differentiation without the need for additional induction reagents (Liu et al. 2019), in the same way that human bone marrow mesenchymal stem cells (hMSCs) attach to it easily during in vitro cell culture, significantly speeding up the early differentiation of hMSCs into osteoblasts (Matsumoto et al. 2015).

8.5.2 Cartilage Regeneration

The primary component of articular cartilage, collagen, is vital to cartilage regeneration because it offers both structural and functional support. Hydrogel or freeze-dried scaffolds are the most common forms of collagen-based biomaterials. They can be enhanced with growth factors or seed cells to create an ideal environment for cartilage tissue regeneration. Hyaline cartilage is primarily composed of Type II collagen (Sophia Fox et al. 2009; Wang et al. 2020). Li et al. (2021) developed injectable cartilage repair hydrogels using collagen and polysaccharides. These hydrogels had beneficial effects on stem cell mobilization and chondrogenic differentiation. Levingstone et al. (2016) created a multi-layer bionic collagen scaffold using large animal models. They inserted it into goat joints to investigate the scaffold's longstanding capacity to mend osteochondral lesions.

8.6 Fish Collagen and Chitosan Mixtures

Biodegradability, biocompatibility, non-toxicity, muco-adhesiveness, antitumor, and antibacterial action are among the utmost sought-after characteristics of chitosan in the medical and cosmetic industries (Sionkowska et al. 2023). Approximately 50–70% of the seafood produced is fish waste, known as side-streams/secondary raw materials for waste valorization. 75% collagen content found in fish bodies is evidence in favour of using fish for collagen production. Skin, scales, fins, head, bones, air bladder, and other internal organs can be used to extract this biopolymer. Fish collagen has a substantially lower denaturation temperature than mammalian collagen because of its lower hydroxyproline concentration (Liu et al. 2014). Collagen, the most abundant protein in the body, is indispensable for preserving the strength and appropriate structure of human tissues as well as for constructing the scaffolds that support internal organs. Chitosan and collagen mixtures acquire unique mechanical and structural qualities over their pure constituents (Sionkowska et al. 2004). The materials derived from the blends exhibit bioresorbability, elasticity, malleability, and more excellent resistance to enzymes than pure collagen. Another significant benefit is the ability to still produce various forms, including

hydrogels, membranes, thin films, and sponges, even after combining the two biopolymers. Because of all these benefits, collagen-chitosan mixtures and the polymers that make them are highly useful in the medical and cosmetic fields. Collagen and chitosan blends can closely resemble the extracellular matrix, which promotes cell division and growth. These characteristics are employed when creating dermal matrices used to treat deep wounds. The manufacture of artificial bones and bone tissue implants makes use of the mixtures' effects on matrix mineralization as well as the growth and variation of osteoblasts (Kaczmarek and Sionkowska 2018; Sharkawy et al. 2021). For the creation of artificial corneas and other tissue engineering applications, chitosan-collagen cross-linked membranes can be employed successfully. These scaffolds can be used for cartilage regeneration and reconstruction after crosslinking from the biopolymers.

Both collagen and chitosan biopolymers are valuable ingredients for a variation of cosmetics owing to their capability to form films. They increase trans epidermal water loss and create a thin layer on the skin's surface, which both enhance the hydration of the epidermis. Hyaluronic acid added to a chitosan-collagen blend can be helpful in hair care products (Sionkowska et al. 2017). Chitosan-collagen hydrogels, which serve as the foundation for various cosmetic products and eliminate the need for preservatives because of chitosan's antibacterial qualities, can be packed with antioxidants, vitamins, or added dynamic components. Chitosan and collagen peptide nanoparticles can be used as emulsion stabilizers, and when combined with the proper additives, chitosan-collagen composites are used to create three-dimensional models of human skin (Sharkawy et al. 2021; Schlotmann et al. 2001).

8.7 Conclusion

In regenerative medicine, collagen is frequently used in procedures like skin repair, cardiovascular repair, and bone/cartilage regeneration. In recent years, tissue engineering and regenerative medicine opt for collagen-based materials over other types of biomaterials on account of their high biocompatibility, low immunogenicity, and structural versatility (Troy et al. 2021). In order to enhance the in vivo effectiveness of collagen-based scaffolds, it is anticipated that forthcoming research endeavours will focus on enhancing their mechanical strength, drug transport capabilities, and biodegradability. Damage to collagen's natural structure during the extraction process is one of the challenges with using it in tissue engineering. This gives rise to poor mechanical abilities and a weak gel-forming competence, which restricts the use of collagen in tissue engineering applications. To enhance collagen's performance and fulfil functional requirements in clinical applications, several forms of collagen including collagen blends, mineralized and crosslinked collagen, and structured collagen are optimized in various ways. Collagen's mechanical qualities and stability can be significantly increased by crosslinking it, but the crosslinking technique must be carefully selected to prevent toxicity and unintentional alterations (Zhu et al. 2022).

Natural polymers with protein and polysaccharide bases exhibit great promise for tissue engineering, drug delivery, and other biomedical uses. Compared to non-mammalian material sources, mammalian-derived polymers are preferred because they have a multitude of physiologically active motifs and favourable host-material responses. It is imperative to evaluate the biochemical and biomechanical properties of starting materials and target tissues in order to comprehend their impact on cell behaviour and tissue formation. Marine collagen derived from aquatic creatures, like fish scales and skin, has recently gained popularity. Marine collagen comes from a sustainable and environmentally friendly source, but further studies are required to realize its unique qualities and uses fully. Developing novel biomaterials that surpass the state-of-the-art options available today regarding inexpensive purification or production costs, fewer side effects and higher therapeutic effectiveness are showing promising trends. Innovating scaffold fabrication techniques in conjunction with ongoing research into new natural biomaterial sources will result in more potent biomaterials and new applications.

References

Ahmed MJ, Hameed BH, Hummadi EH (2020) Review on recent progress in chitosan/chitin-carbonaceous material composites for the adsorption of water pollutants. Carbohydr Polym 247:116690

Anraku M, Fujii T, Kondo Y, Kojima E, Hata T, Tabuchi N et al (2011) Antioxidant properties of high molecular weight dietary chitosan in vitro and in vivo. Carbohydr Polym 83(2):501–505

Aszódi A, Legate KRN, Fässler I, R. (2006) What mouse mutants teach us about extracellular matrix function. Annu Rev Cell Dev Biol 22:591–621

Augst AD, Kong HJ, Mooney DJ (2006) Alginate hydrogels as biomaterials. Macromol Biosci 6(8):623–633

Basa S, Nampally M, Honorato T, Das SN, Podile AR, El Gueddari NE, Moerschbacher BM (2020) The pattern of acetylation defines the priming activity of chitosan tetramers. J Am Chem Soc 142(4):1975–1986

Bello AB, Kim D, Kim D, Park H, Lee SH (2020) Engineering and functionalization of gelatin biomaterials: from cell culture to medical applications. Tissue Eng Part B Rev 26(2):164–180

Bhattarai DP, Aguilar LE, Park CH, Kim CS (2018) A review on properties of natural and synthetic based electrospun fibrous materials for bone tissue engineering. Membranes 8(3):62

Carlström IE, Rashad A, Campodoni E, Sandri M, Syverud K, Bolstad AI, Mustafa K (2020) Cross-linked gelatin-nanocellulose scaffolds for bone tissue engineering. Mater Lett 264:127326

Chang SH, Wu CH, Tsai GJ (2018) Effects of chitosan molecular weight on its antioxidant and antimutagenic properties. Carbohydr Polym 181:1026–1032

Chen FM, Liu X (2016) Advancing biomaterials of human origin for tissue engineering. Prog Polym Sci 53:86–168

Cho YI, No HK, Meyers SP (1998) Physicochemical characteristics and functional properties of various commercial chitin and chitosan products. J Agric Food Chem 46(9):3839–3843

Choi SM, Chaudhry P, Zo SM, Han SS (2018) Advances in protein-based materials: from origin to novel biomaterials. In: Cutting-edge enabling technologies for regenerative medicine, pp 161–210

Coppola D, Oliviero M, Vitale GA, Lauritano C, D'Ambra I, Iannace S, de Pascale D (2020) Marine collagen from alternative and sustainable sources: extraction, processing and applications. Mar Drugs 18(4):214

Cui B, Zhang C, Gan B, Liu W, Liang J, Fan Z, Zhou Y (2020) Collagen-tussah silk fibroin hybrid scaffolds loaded with bone mesenchymal stem cells promote skin wound repair in rats. Mater Sci Eng C 109:110611

de Alvarenga ES, de Oliveira CP, Bellato CR (2010) An approach to understanding the deacetylation degree of chitosan. Carbohydr Polym 80(4):1155–1160

Dimitriou R, Jones E, McGonagle D, Giannoudis PV (2011) Bone regeneration: current concepts and future directions. BMC 9(1):1–10

Dong C, Lv Y (2016) Application of collagen scaffold in tissue engineering: recent advances and new perspectives. Polymers 8(2):42

Ghomi F, Daliri M, Godarzi V, Hemati M (2021) A novel investigation on the characterization of bioactive glass cement and chitosan-gelatin membrane for jawbone tissue engineering. J Nanoanalysis 8(4):292–301

Han Y, Jiang Y, Hu J (2020) Tea-polyphenol treated skin collagen owns coalesced adaptive-hydration, tensile strength and shape-memory property. Int J Biol Macromol 158:1–8

Hashmi MP, Koester TM (2018) Applications of synthetically produced materials in clinical medicine. In: Reference module in materials science and materials engineering. Elsevier. (in press). https://doi.org/10.1016/B978-0-12-803581-8.10258-9

Howling GI, Dettmar PW, Goddard PA, Hampson FC, Dornish M, Wood EJ (2001) The effect of chitin and chitosan on the proliferation of human skin fibroblasts and keratinocytes in vitro. Biomaterials 22(22):2959–2966

Hu CH, Yao CH, Chan TM, Huang TL, Sen Y, Huang CY, Ho CY (2016) Effects of different concentrations of collagenous peptide from fish scales on osteoblast proliferation and osteoclast resorption. Chin J Physiol 59:191–201

Inamdar NN, Mourya V (2014) Chitosan and low molecular weight chitosan: biological and biomedical applications. Adv Biomater Biodev:183–242

Islam S, Bhuiyan MR, Islam MN (2017) Chitin and chitosan: structure, properties and applications in biomedical engineering. J Polym Environ 25:854–866

Jankangram W, Chooluck S, Pomthong B (2016) Comparison of the properties of collagen extracted from dried jellyfish and dried squid. Afr J Biotechnol 15(16):642–648

Joyce K, Fabra GT, Bozkurt Y, Pandit A (2021) Bioactive potential of natural biomaterials: identification, retention and assessment of biological properties. Signal Transduct Target Ther 6(1):122

Kaczmarek B, Sionkowska A (2018) Chitosan/collagen blends with inorganic and organic additive—a review. Adv Polym Technol 37(6):2367–2376

Khan R, Khan MH (2013) Use of collagen as a biomaterial: an update. J Indian Soc Periodontol 17(4):539

Klemm D, Schumann D, Kramer F, Heßler N, Koth D, Sultanova B (2009). Nanocellulose materials–different cellulose, different functionality. In: Macromolecular symposia, vol 280, no 1. Wiley-VCH Verlag, Weinheim, pp 60–71

Kumari S, Annamareddy SHK, Abanti S, Rath PK (2017) Physicochemical properties and characterization of chitosan synthesized from fish scales, crab and shrimp shells. Int J Biol Macromol 104:1697–1705

Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. Prog Polym Sci 37(1):106–126

Lee BH, Lum N, Seow LY, Lim PQ, Tan LP (2016) Synthesis and characterization of types A and B gelatinmethacryloyl for bioink applications. Materials 9(10):797

Letchford K, Södergard A, Plackett D, Gilchrist SE, Burt HM (2011) Lactide and glycolide polymers. In: Biodegradable polymers in clinical use and clinical development, pp 317–365

Levingstone TJ, Ramesh A, Brady RT, Brama PA, Kearney C, Gleeson JP, O'Brien FJ (2016) Cell-free multi-layered collagen-based scaffolds demonstrate layer specific regeneration of functional osteochondral tissue in caprine joints. Biomaterials 87:69–81

Li Z, Cao H, Xu Y, Li X, Han X, Fan Y, Zhang X (2021) Bioinspired polysaccharide hybrid hydrogel promoted recruitment and chondrogenic differentiation of bone marrow mesenchymal stem cells. Carbohydr Polym 267:118224

Lim YS, Ok YJ, Hwang SY, Kwak JY, Yoon S (2019) Marine collagen as a promising biomaterial for biomedical applications. Mar Drugs 17(8):467
Lin N, Dufresne A (2014) Nanocellulose in biomedicine: current status and future prospect. Eur Polym J 59:302–325
Liu D, Zhou P, Li T, Regenstein JM (2014) Comparison of acid-soluble collagens from the skins and scales of four carp species. Food Hydrocoll 41:290–297
Liu X, Zheng C, Luo X, Wang X, Jiang H (2019) Recent advances of collagen-based biomaterials: multi-hierarchical structure, modification and biomedical applications. Mater Sci Eng C 99:1509–1522
Lodhi G, Kim YS, Hwang JW, Kim SK, Jeon YJ, Je JY et al (2014) Chitooligosaccharide and its derivatives: preparation and biological applications. BioMed Res Int 2014:654913
Magadala P, Amiji M (2008) Epidermal growth factor receptor-targeted gelatin-based engineered nanocarriers for DNA delivery and transfection in human pancreatic cancer cells. Am Assoc Pharm Scient J 10:565–576
Matsumoto R, Uemura T, Xu Z, Yamaguchi I, Ikoma T, Tanaka J (2015) Rapid oriented fibril formation of fish scale collagen facilitates early osteoblastic differentiation of human mesenchymal stem cells. J Biomed Mater Res A 103(8):2531–2539
Nair R, Sevukarajan M, Mohammed T, Badivaddin CK, Kumar A (2010) Collagen based drug delivery systems: a review. J Innov Pharm Sci 1:288–304
Naveed M, Phil L, Sohail M, Hasnat M, Baig MMFA, Ihsan AU et al (2019) Chitosan oligosaccharide (COS): an overview. Int J Biol Macromol 129:827-843
Narayanan D, Geena MG, Lakshmi H, Koyakutty M, Nair S, Menon D (2013) Poly-(ethylene glycol) modified gelatin nanoparticles for sustained delivery of the anti-inflammatory drug Ibuprofen-Sodium: An in vitro and in vivo analysis. Nanomedicine 9:818–828
NIBIB (2023) Biomaterials. National Institute of Biomedical Imaging & Bioengineering, US Department of Health and Human Services. https://www.nibib.nih.gov/science-education/science-topics/biomaterials
No HK, Meyers SP (1995) Preparation and characterization of chitin and chitosan—a review. J Aqua Food Product Technol 4(2):27–52
Normand V, Lootens DL, Amici E, Plucknett KP, Aymard P (2000) New insight into agarose gel mechanical properties. Biomacromolecules 1(4):730–738
Parenteau-Bareil R, Gauvin R, Berthod F (2010) Collagen-Based Biomaterials for Tissue Engineering Applications. Materials (Basel) 16;3(3):1863–87
Prang P, Müller R, Eljaouhari A, Heckmann K, Kunz W, Weber T, Weidner N (2006) The promotion of oriented axonal regrowth in the injured spinal cord by alginate-based anisotropic capillary hydrogels. Biomaterials 27(19):3560–3569
Rasweefali MK, Sabu S, Sunooj K, Sasidharan A, Xavier KM (2021) Consequences of chemical deacetylation on physicochemical, structural and functional characteristics of chitosan extracted from deep-sea mud shrimp. Carbohydr Polym Technol Appl 2:100032
Rasweefali MK, Sabu S, Azad KM, Rahman MR, Sunooj KV, Sasidharan A, Anoop KK (2022) Influence of deproteinization and demineralization process sequences on the physicochemical and structural characteristics of chitin isolated from deep-sea mud shrimp (Solenocerahextii). Adv Biomarker Sci Technol 4:12–27
Rinaudo M (2008) Main properties and current applications of some polysaccharides as biomaterials. Polym Int 57(3):397–430
Rodríguez-Vázquez M, Vega-Ruiz B, Ramos-Zúñiga R, Saldaña-Koppel DA, Quiñones-Olvera LF (2015) Chitosan and its potential use as a scaffold for tissue engineering in regenerative medicine. Biomed Res Int 2015:1–15
Sabu S, Sasidharan A, Venugopal V (2022) Influence of isolation conditions on the physicochemical and biological properties of chitosan and chitosan oligosaccharides from marine crustacean shell wastes. In: Chitooligosaccharides: prevention and control of diseases. Springer International Publishing, Cham, pp 333–352

Schlotmann K, Kaeten M, Black AF, Damour O, Waldmann-Laue M, Förster T (2001) Cosmetic efficacy claims in vitro using a three-dimensional human skin model. Int J Cosmet Sci 23(5):309–318

Sharip NS, Ariffin H (2019) Cellulose nanofibrils for biomaterial applications. Mater Today Proc 16(4):1959–1968

Sharkawy A, Barreiro MF, Rodrigues AE (2021) New Pickering emulsions stabilized with chitosan/collagen peptides nanoparticles: synthesis, characterization and tracking of the nanoparticles after skin application. Colloids Surf A Physicochem Eng Asp 616:126327

Shirvan AR, Shakeri M, Bashari A (2019) Recent advances in application of chitosan and its derivatives in functional finishing of textiles. In: The impact and prospects of green chemistry for textile technology. Elsevier, pp 107–133

Singh BN, Pramanik K (2018) Generation of bioactive nano-composite scaffold of nanobioglass/silk fibroin/carboxymethyl cellulose for bone tissue engineering. J Biomater Sci Polym Ed 29(16):2011–2034

Sionkowska A, Wisniewski M, Skopinska J, Kennedy CJ, Wess TJ (2004) Molecular interactions in collagen and chitosan blends. Biomaterials 25(5):795–801

Sionkowska A, Kaczmarek B, Michalska M, Lewandowska K, Grabska S (2017) Preparation and characterization of collagen/chitosan/hyaluronic acid thin films for application in hair care cosmetics. Pure Appl Chem 89(12):1829–1839

Sionkowska A, Lewandowska K, Kurzawa M (2023) Chitosan-based films containing rutin for potential cosmetic applications. Polymers 15(15):3224

Smallman RE, Bishop RJ (1999) Modern physical metallurgy and materials engineering. Butterworth-Heinemann

Sood A, Gupta A, Bharadwaj R, Ranganath P, Silverman N, Agrawal G (2022) Biodegradable disulfide crosslinked chitosan/stearic acid nanoparticles for dual drug delivery for colorectal cancer. Carbohydr Polym 294:119833

Sophia Fox AJ, Bedi A, Rodeo SA (2009) The basic science of articular cartilage: structure, composition, and function. Sports Health 1(6):461–468

Sun J, Tan H (2013) Alginate-based biomaterials for regenerative medicine applications. Materials 6(4):1285–1309

Sun W, Liu W, Wu Z, Chen H (2020) Chemical surface modification of polymeric biomaterials for biomedical applications. Macromol Rapid Commun 41(8):1900430

Swiatkiewicz S, Swiatkiewicz M, Arczewska-Wlosek A, Jozefiak D (2015) Chitosan and its oligosaccharide derivatives (chito-oligosaccharides) as feed supplements in poultry and swine nutrition. J Anim Physiol Anim Nutr 99(1):1–12

Tarafder S, Lee CH (2016) Synovial joint: *in situ* regeneration of osteochondral and fibrocartilaginous tissues by homing of endogenous cells. In: *In situ* tissue regeneration. Academic Press, pp 253–273

Tekale SU, Kanagare AB, Dhirbassi AV, Domb AJ, Pawar RP (2022) Polysaccharide-based biomaterials: overview, Biomaterials science series. Royal Society of Chemistry, p 580

Tielens S, Declercq H, Gorski T, Lippens E, Schacht E, Cornelissen M (2007) Gelatin-based microcarriers as embryonic stem cell delivery system in bone tissue engineering: an in-vitro study. Biomacromolecules 8(3):825–832

Troy E, Tilbury MA, Power AM, Wall JG (2021) Nature-based biomaterials and their application in biomedicine. Polymers 13(19):3321

Ullah S, Chen X (2020) Fabrication, applications and challenges of natural biomaterials in tissue engineering. Appl Mater Today 20:100656

Van der Lubben IM, Kersten G, Fretz MM, Beuvery C, Verhoef JC, Junginger HE (2003) Chitosan microparticles for mucosal vaccination against diphtheria: oral and nasal efficacy studies in mice. Vaccine 21(13–14):1400–1408

Wang Q, Li X, Wang P, Yao Y, Xu Y, Chen Y, Zhang X (2020) Bionic composite hydrogel with a hybrid covalent/noncovalent network promoting phenotypic maintenance of hyaline cartilage. J Mater Chem B 8(20):4402–4411

Xu N, Peng XL, Li HR, Liu JX, Cheng JSY, Qi XY et al (2021) Marine-derived collagen as biomaterials for human health. Front Nutr 8:702108

Younes I, Rinaudo M (2015) Chitin and chitosan preparation from marine sources. Structure, properties and applications. Mar Drugs 13(3):1133–1174

Zang S, Zhang R, Chen H, Lu Y, Zhou J, Chang X, Yang G (2015) Investigation on artificial blood vessels prepared from bacterial cellulose. Mater Sci Eng C 46:111–117

Zhang D, Wu X, Chen J, Lin K (2018) The development of collagen based composite scaffolds for bone regeneration. Bioactive Mater 3(1):129–138

Zheng Z, Li M, Shi P, Gao Y, Ma J, Li Y, Yang L (2021) Polydopamine-modified collagen sponge scaffold as a novel dermal regeneration template with sustained release of platelet-rich plasma to accelerate skin repair: a one-step strategy. Bioactive Mater 6(8):2613–2628

Zhou Y, Yang D, Chen X, Xu Q, Lu F, Nie J (2008) Electrospun water-soluble carboxyethyl chitosan/poly (vinyl alcohol) nanofibrous membrane as potential wound dressing for skin regeneration. Biomacromolecules 9(1):349–354

Zhu J, Li Z, Zou Y, Lu G, Ronca A, D'Amora U, Liang J, Fan Y, Zhang, Sun, Y (2022) Advanced application of collagen-based biomaterials in tissue repair and restoration. Journal of Leather Science and Engineering 4(30)