

# Fish Muscle

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Fish muscle, which accounts for 15%–25% of the total protein in fish, is a desirable protein source. Their hydrolysate is in high demand nutritionally as a functional food and thus has high potential added value. The hydrolysate contains physiologically active amino acids and various essential nutrients, the contents of which depend on the source of protein, protease, hydrolysis method, hydrolysis conditions, and degree of hydrolysis.

Keywords: fish muscle ; amino acid ; protein hydrolysate ; enzyme ; bioactivity ; human health

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## 1. Introduction

The use of fish and seafood products, including aquatic plants, has increased by ~8% over the last 30 years, with high growth rates of global livestock production <sup>[1]</sup>. From 1986 to 2016, the annual consumption of fishery products increased from 71.8 million tons to 148.2 million tons, followed by 156.4 million tons in 2018, due to a continuous supply of fish for human consumption through the development of aquaculture and the allowance of captive fisheries <sup>[2]</sup>. Between 1961 and 2018, the average annual rise in global food fish consumption of 3.1% had outpaced the population growth (1.6%) and exceeded the consumption escalation of all terrestrial animal products combined (such as beef, poultry, and milk), which increased by 2.1% per annum <sup>[2][3]</sup>. In particular, the global fishery market in 2030 is expected to increase by 18% compared to 2018 due to the continuous increase in fishery production, income increase due to urbanization, and changes in eating habits <sup>[2]</sup>.

Various clinical studies have identified the health-promoting effects of consuming fish proteins. This suggests that fish consumption reduces the risk of cardiovascular diseases among elderly persons. (2017) found that among Japanese persons aged 40–59 years in 1990 and 40–69 years in 1993, fish consumption equivalent to 111 mg of eicosapentaenoic acid (EPA) or 123 mg of docosahexaenoic acid (DHA) per day was associated with a reduced risk of major depressive disorder <sup>[4]</sup>. They noted that further investigation is needed to determine whether lean fish itself protects against type 2 diabetes, or whether lean fish consumption affects consumer lifestyles <sup>[5]</sup>.

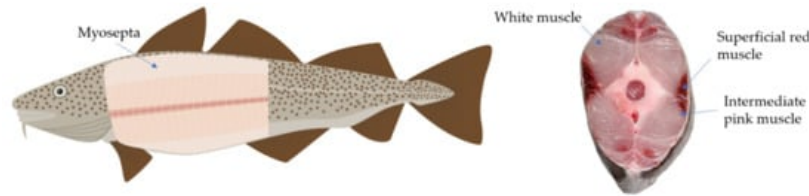
Fish muscle, which contains 15%–25% of total protein in fish, can be divided into myofibrillar (50%–60%), sarcoplasmic (30%), and stromal (10%–20%) proteins <sup>[6]</sup>. Therefore, efforts have been directed toward full absorption of essential nutrients and amino acids present in fish by hydrolyzing fish proteins. In addition, in terms of their natural availability, relatively low-cost extraction methods, and ability to exert beneficial effects on human health, fish muscle protein hydrolysates and their amino acids are desirable functional ingredients <sup>[7]</sup>. This review examines the status of recent research findings on fish protein hydrolysates and their proximal components, amino acid composition, and functional and biological properties.

## 2. Characteristics of Fish Muscle Protein

Human food is produced from protein that comprises ~60% of the body weight of fish. Skeletal muscle proteins in fish facilitate muscle movement by contraction. The heterogeneous fibrous populations of cells in fish proteins differ in terms of molecular, structural, contractile, and metabolic functions and influence the growth rate and properties of muscle, such as the onset of firmness, pH decline, color, and cohesiveness, and ultimately the functional properties of meat <sup>[8][9][10]</sup>. The firmness of muscle varies with cellularity, including muscle fiber density and diameter; therefore, the flesh of fish is softer than that of terrestrial animals as constant energy is not needed to support their skeletons in water <sup>[11]</sup>.

In contrast to terrestrial animals, fish have shorter muscle fibers and less connective tissue, and these muscles are segmented into myotomes by fine connective tissue layers called myocommata or myosepta, which lie primarily in thin sheets that separate muscle fibers into orderly layers <sup>[12][13]</sup>. In addition, muscle fiber in fish is categorized into three main types of muscle, namely a major white muscle, a superficial red muscle, and an intermediate pink muscle; the axial muscle consists mainly of fast white fibers, covered by a thin layer of slow-red muscle fibers at the periphery, with a layer

of pink intermediate muscle fibers in between them (**Figure 1**, [14]). Salmonids lack the intermediate fiber type. Although the connective tissue content is low but more evenly distributed in muscles of fish than in terrestrial animals, firmness increases along the anterior–posterior axis of the filet.



**Figure 1.** Whole fish and sectional slide with myosepta, major white muscle, a superficial red muscle, and an intermediate pink muscle.

Muscle proteins from fish are easily digestible and rich in more essential amino acids than most terrestrial meat proteins [3]. The consumption of fish muscle protein is associated with health benefits, particularly anti-inflammatory, antioxidant, and angiotensin-converting enzyme inhibitory activities and antimicrobial effects [15][16][17][18][19][20]. Hydrolysates of fish muscle proteins with good nutritional composition, amino acid profiles, and biological activities are easily absorbed in the gastrointestinal tract, and their bioavailability and stability due to decreased fragments have attracted attention in terms of producing valuable food ingredients. Current knowledge of muscle protein hydrolysates and their amino acid composition and biological activities in various fish are reviewed below.

### 3. Fish Muscle Protein Hydrolysates

Fish protein hydrolysates are produced by chemical hydrolysis with either alkali or acid [21][22], fermentation with proteolytic microorganisms [23][24], or by digestion with enzymes [25][26][27][28].

Alkaline or acid hydrolysis results in reduced consumer acceptance due to the high variability of hydrolysates because the chemical cleavage of peptide bonds is not specific. Hydrolysis damages amino acid profiles by oxidizing cysteine and methionine, destroying some serine and threonine residues, and possibly converting asparagine and glutamine to aspartate and glutamate [29].

Fermentation with proteolytic microorganisms can enhance the nutritive and bioactive characteristics of fish matrices and improve human health benefits via the enzymatic activity of the raw material and the metabolic activity of microorganisms [24]. For instance, the beneficial aspects of consuming fermented fish protein products include antioxidant [30], ACE-inhibitory [31], anticancer [32], and antibacterial properties [30]. Ealier et al. found that fermentation causes a gradual decrease in the content of salt-soluble and water-soluble proteins in carp sausage, while increasing the content of free amino acids and insoluble proteins [33]. The advantage of fermentation is that microbial proteases hydrolyze the protein into bioactive peptides that can be purified without further hydrolysis.

Enzymatic hydrolysis generates specific peptides by cleaving site-specific chains according to the specificity of the enzyme; this allows more efficient control of the manufacturing process by easily inactivating the enzyme after a specific degree of hydrolysis (DH) has been achieved [34]. The composition and subsequent physiological activities of the resulting hydrolysates are also highly influenced by the protein source, method and conditions of hydrolysis, and DH [35][36]. Thus, enzymatic hydrolysis is the most popular method for producing bioactive protein hydrolysates. The functional properties of protein hydrolysates have been improved using gut extracts from catfish, squid, sleek hound, gray trigger, golden mullet, and goby.

1shows the various fish muscle protein hydrolysates used in proteolysis. Several protein hydrolysates are currently being produced from muscle proteins of monkfish ([37]), red lionfish (*Pterois volitans* L., [17]), cobia (*Rachycentron canadum*, [38]), catfish [39], skipjack tuna (*Katsuwonus pelamis*, [40]), bighead croaker (*Collichthys niveatus*, [41]), Pacific whiting (*Merluccius productus*, [42]), ornate threadfin bream (*Nemipterus hexodon*, [43]), brownstripe red snapper (*Lutjanus vitta*, [44]), cuttlefish (*Sepia officinalis*, [45]), salmon (*Salmon salar*, [46]), sardinelle (*Sardina pilchardus*, [47]), thornback ray fish (*Raja clavate*, [48]), Pipefish

A comparison of cobia flesh hydrolyzed by Alcalase, Flavourzyme, and Protamex found that hydrolysates generated by Protamex have a higher DH and significant amounts of free tyrosine [38]. hydrolyzed bighead croaker muscle protein using Alcalase or Neutrase under specific temperature, pH, and enzyme-to-substrate ratios following degrees of hydrolysis and determined levels of sweet and umami amino acids produced from the hydrolysates of muscle protein in a study of response surface methodology [41]. Khantaphant, Benjakul, and Kishimura (2011) generated protein hydrolysates with

40% DH from brownstripe red snapper muscle using Alcalase or Flavourzyme as a first step, followed by hydrolysis with pyloric caeca protease (isolated from brownstripe red snapper muscle) as the second step [44]. Enzyme extracts from the digestive tract, namely the stomach, pyloric caeca, and intestine, have also been applied to prepare protein hydrolysates from fish muscles and characterize their functional properties [43][45][46].

## 4. Amino Acids in Fish Protein Hydrolysates

Proteolytic enzymes break down proteins into hydrolysates that comprise small peptides consisting of 2–20 amino acids [49]. The molecular weight, length, and sequence of the peptides and their amino acid composition influence their bioactive properties; hydrolysates produce the forms of amino acids that are useful in supporting various human biological functions [50][51]. The amino acid composition of food proteins plays an important role in various human physiological activities and directly or indirectly affects the maintenance of human health. Amino acids are essential for the synthesis of various proteins with important functions, including oxygen carriers, vitamins, CO<sub>2</sub>, enzymes, and structural proteins

Tyrosine, methionine, histidine, lysine, and tryptophan have powerful radical scavenging activity in oxidative reactions [52]. Histidine significantly enhances antioxidant capacity because the protonation of the imidazole ring acts as a hydrogen donor [53]. The aromatic amino acids phenylalanine, tryptophan, and tyrosine possess considerable antioxidant activity and powerful chelating effects [54]. Glutamic and aspartic acids have potential antiproliferative activity against tumor cells [55], and low concentrations of aromatic amino acids rich in glycine and proline have more potent 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, possibly due to nonaromatic amino acids, such as glycine and proline [56].

Fish proteins are easily digested and abundant in essential amino acids that are limited in terrestrial meat proteins such as methionine and lysine (6.5% vs. 5.7% and 19.6% vs. 19.0% of total essential amino acids in fish vs. terrestrial meat, respectively) [1]. Zou et al. (2016) tracked preferential amino acids for antioxidant activities among selected antioxidant peptides [52] and found that 33.7% of total amino acids were glycine, proline, and leucine; 18.7% were alanine, tyrosine, and valine; and 4.9% were methionine, glutamine, and cysteine. They emphasized that a high proportion of hydrophobic amino acids confers the ability of peptides to scavenge radicals.

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