Chapter 6

Nutritional significance of fish proteins

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Fish is a vital source of food for people and it plays a major role in human nutrition. Importance of fish as a source of high quality, balanced and easily digestible protein is now well understood. It is man's most important single source of high-quality protein, providing ~16% of the animal protein consumed by the world's population, according to the Food and Agriculture Organization (FAO) of the United Nations (1997). It is a particularly important protein source in regions where livestock is relatively scarce-fish supplies <10% of animal protein consumed in North America and Europe, but 17% in Africa, 26% in Asia and 22% in China (FAO, 2000). The FAO estimates that about one billion people world-wide rely on fish as their primary source of animal protein (FAO, 2000). In general, people in developing countries are much more dependent on fish as part of their daily diets than those living in the developed world. The world's oceans, lakes and rivers are harvested by artisanal fishers who provide vital nourishment for poor communities, in Africa and Asia, in many parts of Latin America and islands in the Pacific and Indian oceans. Of the 30 countries most dependent on fish as a protein source, all but four are in the developing world. Besides, it is also a well-known source of several other nutrients and is being accepted as a healthy food. A health food should contain all the essential nutrients *viz*., carbohydrates, proteins, fats and minerals and vitamins in right proportions and in this context, fish assumes significance Understandably, the per capita fish consumption is increasing considerably world over.

Understanding the composition of food is very important to determine its nutritive value. This is particularly true for commodities like fish as there is large variation in the composition. There are group specific and species-specific changes in chemical composition.

Composition varies depending on age, sex, environment and season. The variation is also closely related to feed intake, migratory swimming and sexual changes in connection with spawning. Fish have starvation periods for natural or physiological reasons (such as migration and spawning) or because of external factors such as shortage of food. Spawning, whether occurring after long migrations or not, usually requires higher levels of energy. In fact, fish with energy depots in the form of lipids rely on this. Species migrating long distances before reaching specific spawning grounds or rivers may use proteins in addition to lipids for energy, depleting both lipid and protein reserves and generally reducing their biological condition. In addition, most species do usually not ingest much food during spawning migration and are therefore not able to supply energy through feeding. Fish are highly heterogeneous group with over 28,000 species. Fish are available from marine, freshwater and brackishwater environments. The composition of fish living in marine environment is different in certain aspects from that from freshwater and brackishwater environments.

Fish is a food of excellent nutritional value and in addition to providing high quality, easily digestible protein, it is also a good source of a wide variety of vitamins and minerals, including vitamins A and D, phosphorus, magnesium, selenium, and iodine in marine fish. Even in small quantities, fish protein can have a significant positive impact in improving the quality of dietary protein by complementing the essential amino acids (lysine and sulphur-containing amino acids) that are often present in low quantities in vegetable-based diets like cereals and legumes that are typically consumed in many developing countries. Fish protein can be used therefore to complement the amino acid pattern and improve the overall protein quality of a mixed diet. Fish contain both macro and micro nutrients. Moisture, fat, protein and minerals are the main components of fish meat and the analysis of the same is referred to as 'proximate composition'. The chemical composition of fish is closely related to feed intake, migratory swimming and sexual changes in connection with spawning. Fish have starvation periods for natural or physiological reasons such as migration and spawning or because of shortage of food. Nutritionally, fish provide mainly protein, with 80-90% of food energy derived from this. They contain relatively low fat, and carbohydrate is virtually absent in all bony fish commonly eaten. Carbohydrates, vitamins, nucleotides and other non-protein nitrogenous constituents are present in minor quantities even though some of them are playing a vital role in maintaining the system and thus essential for growth and development.

The table below shows the principle components of fish as well as their variation. The average composition of beef muscle has been included for comparison.

Constituent	Fish fillet			Beef muscle
	Minimum	Normal variation	Maximum	
Protein	6	16-21	28	20
Lipid	0.1	0.2-25	67	3
Carbohydrates		<0.5		1
Ash	0.4	1.2-1.5	105	1
Water	28	66-81	96	75

Table 1. Principle constituents of fish and beef muscle (in percentage)

Water is the most abundant substance in living systems, making up 70% or more of the weight of most organisms. Water pervades all portions of every cell and is the medium in which the transport of nutrients, cytoplasmic reactions for the maintenance of cells and the transfer of chemical energy occur. The proportion of water in fish varies widely between 65-90%, although it is normally in the range of 70-75%. There exists an inverse relationship between water and fat content. Low water content is usually associated with relatively high fat content and vice-versa. An example of a species with high water content is Bombay duck (*Harpadon nehereus*), with about 90% water in the flesh.

In fish, water is present in two forms - bound to the proteins and free. These forms have well defined biological roles but not of much significance as food. Water is lost from the tissue in many ways during processing and this may affect the quality, especially the texture. The water in fresh fish muscle is tightly bound to the proteins in the structure in such a way that it cannot readily be expelled even under high pressure. After prolonged chilled or frozen storage, however, the proteins losses the capacity to hold water due to denaturation effects and water is lost as drip. In the living fish, the water content usually increases and the protein content decreases as spawning approaches. During different seasons with increase in fat content, the water content decreases.

Protein

Proteins are perhaps the most important biomolecules and are made up of amino acids, joined together by peptide bonds and have versatile role in the living systems. The protein content of fish muscle ranges between 16 and 21%. Protein content below 15% signifies low protein. The protein content varies with the type of muscle, and dark muscles usually contain low levels of moisture and protein compared to light muscle. Proteins are complex molecules and the molecular weight of proteins range from less than 5000 dalton to several thousand daltons.

Proteins are generally classified based on the shape, solubility and chemical structure. Based on the solubility in salt solutions, proteins are classified into three groups.

- ***Structural or myofibrillar proteins*** (actin, myosin, tropomyosin and troponin). Structural proteins, which constitute 65-70 % of the total protein (compared with 40% in mammals).

These proteins are soluble in neutral salt solutions of high ionic strength (0.5 M). The structural proteins make up the contractile apparatus responsible for the muscle movement. The amino-acid composition is roughly similar to corresponding proteins in mammalian muscle, although the physical properties can differ slightly. The rheological and functional properties of fish proteins are associated with these proteins and play a significant role in the preparation of surimi based products.Changes in the environment alter the nature of these fish proteins. Treatment with high salt concentrations or heat may lead to denaturation, irreversibly changing the native protein. When the proteins are denatured under controlled conditions, their properties may be utilized for technological purposes like processing of fish for product development. A good example is the production of surimi-based products in which the gel-forming ability of the myofibrillar proteins is used. After salt and stabilizers are added to a washed, minced preparation of muscle proteins and after a controlled heating and cooling procedure, the proteins form a very strong gel.

- ***Sarcoplasmic proteins*** (myoalbumin, globulin and enzymes) which are soluble in neutral salt solutions of low ionic strength (<0.15 M). In fish meat this fraction constitutes 25-30% of protein. In comparison, mammalian meat contains about 35 – 40% sarcoplasmic proteins. Among fish, the pelagic fish (sardines, mackerel etc.) have higher content of sarcoplasmic proteins while demersal fish (flat fishes, sciaenids etc.) have lower content. The sarcoplasmic protein fraction contains metabolic enzymes localized inside the cytoplasm or inside cell organelles like endoplasmic reticulum, mitochondria and lysosomes. As there is uniqueness associated with sarcoplasmic proteins from different species, the electrophoretic patterns of these fractions can be used for species identification. The majority of sarcoplasmic proteins are enzymes participating in cell metabolism, such as the anaerobic energy conversion from glycogen to ATP. If the organelles within the muscle cells are broken, this protein fraction may also contain the metabolic enzymes localized inside the endoplasmatic reticulum, mitochondria and lysosomes.

  The fact that the composition of the sarcoplasmic protein fraction changes when the organelles are broken was suggested as a method for differentiating fresh from frozen fish, under the assumption that the organelles were intact until freezing. However, it was later stated that these methods should be used with great caution as some of the enzymes are liberated from the organelles during iced storage of fish as well.

- ***Connective tissue or stroma proteins*** (collagen): Connective tissue proteins constitute approximately 3% of the protein in teleosts and about 10% in elasmobranchs (compared to about 17% in mammals). It is this low content of collagen that gives the soft texture to fish meat. Stroma proteins are insoluble in neutral salt solution or in dilute acids or alkalis. Collagen is the major connective tissue protein in fish. The chemical and physical properties of collagen proteins are different in tissues such as skin, swim bladder and the myocommata in muscle. Collagen fibrils form a delicate network structure with varying complexity in the different connective tissues in a pattern similar to that found in mammals. However the collagen in fish is much more thermolabile and contains fewer but more labile cross-links than collagen from warm-blooded vertebrates. The hydroxyproline content is in general lower in fish than in other animals. Different fish species contain varying amounts of collagen in the body tissues. This has led to a theory that the distribution of collagen may reflect the swimming behaviour of the species. Further, the varying amounts and types of collagen in different fishes may also have an influence on the textural properties of fish muscle.

- Fish proteins contain all the essential amino acids (not synthesized and need to be provided in the diet) in the required proportion and hence have a high nutritional value, which contribute to their high biological value (Table 2). Cereal proteins are usually low in lysine and/or the sulphur-containing amino acids like methionine and cysteine, whereas fish protein is an excellent source of these amino acids. In diets based mainly on cereals, a supplement of fish can, therefore, raise the biological value significantly. The chemical score or amino acid score of fish protein (70) compares well with that of whole egg protein (69), which is considered a standard protein source, and slightly more than that of cow's milk (60). Similarly the protein efficiency ratio of fish proteins is 3.5 against that of egg protein (3.9), beef (2.3) and milk protein (2.5). Fish is also rich in the non-protein amino acid taurine, which has a unique role in neurotransmission.

Amino acid	Fish	Milk	Beef	Eggs
Lysine	8.8	8.1	9.3	6.8
Tryptophan	1.0	1.6	1.1	1.9
Histidine	2.0	2.6	3.8	2.2
Phenyl alanine	3.9	5.3	4.5	5.4
Leucine	8.4	10.2	8.2	8.4
Isoleucine	6.0	7.2	5.2	7.1
Threonine	4.6	4.4	4.2	5.5
Methionine-cystine	4.0	4.3	2.9	3.3
Valine	6.0	7.6	5.0	8.1

Table 2. Essential amino-acids (percentage) in various proteins

Cereal grains are usually low in lysine and/or sulphur-containing amino acids (methionine and cysteine), whereas fish protein is an excellent source of these amino acids. A supplement of fish can therefore significantly raise the biological value in cereal-based diets. In addition to the fish proteins already mentioned there is a renewed interest in specific protein fractions that can be recovered from by-products, particularly in the viscera. One such example is the basic protein or protamines found in the milt of the male fish, which can contain as much as 65 percent arginine. The best sources are salmonids and herring, whereas ground fish such as cod are not found to contain protamines. The extreme basic character of protamines makes them interesting for several reasons. As they adhere to most other proteins less basic, they can enhance the functional properties of other food proteins (if all the lipids present in the milt are removed from the protein preparation to avoid off-flavour development in the finished products). But the most promising feature of basic proteins is their ability to prevent growth of microorganisms.

Much less is known about the clinical effects of fish protein. There are, however, several indications, from e.g. studies on animals, about fish protein's health effects. Fish proteins have been suggested to prevent from insulin resistance in high-fat fed obese rats to improve glucose-insulin metabolism and dyslipidemia and diminish development of high blood pressure and hypercholesterolemia.

Spoilage

Spoilage changes affect the quality of the material and are associated with handling issues. Following death, the circulation of blood to body ceases leading to depletion of oxygen supply to the tissues. This results in the inability of body tissues to synthesis ATP as Electro Transport Chain (ETC) and oxidative phosphorylation mechanisms are no longer operative. This results in the depletion of ATP and creatine phosphate. Alongside this, the anaerobic conversion of glucose to lactic acid leads to drop in pH. This accelerates rigor mortis and protein denaturation. The drop in pH accelerates action of cathepsins and other proteolytic enzymes. This leads to accumulation of various metabolites, flavour, bacterial growth and ultimately spoilage. These changes get quickened if the conditions of storage are not proper. The muscle myofibrillar protein particularly actin and myosin changes in relation to rigor mortis. The actin and myosin are dissociated in pre-rigor stage. Depletion of ATP gradually associates the two leading to the formation of actomyosin. The fish sarcoplasmic proteins are far more stable than myofibrillar protein. They possess better thermostability and solubility than their counterparts in other meats and do not appear to play roles in muscle texture.

Processing

With increasing demand for fish for food, storing fish for future use is important. The conditions like chill or frozen storage prolong the changes in composition. But long term storage after freezing affects the quality of proteins, thereby affecting functional property of proteins and hence the quality of the fish. This takes different rates in the case of fatty fishes. On the other hand thermal processing causes abrupt changes and the functionality of the product gets affected. Protein in native 3D structure gets affected if the environment changes. The protein structure opens up exposing the hydrophobic residues to the exterior. These favour interaction between other food components particularly the lipids and the functionality gets altered, for good or bad of the product. Therefore knowledge on these lines is essential to make a protocol for value addition of fish and shellfish.