

## Biochemical Composition of fish

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Fish is considered to be a valued item of food by a great majority. This acceptance of fish as a preferred food is due to its special culinary and nutritional properties. But it is of common knowledge that all varieties of fish do not have the same properties. The taste, flavour and nutritional value of fish vary considerably from species, to species and even among the same species individual variations can be observed. What is the reason for such variations? The answer to this question lies in the fact that the properties of fish – culinary, physical or nutritional – depend on its biochemical composition and the biochemical composition is not the same for all species.

It is the muscle tissue of fish that is used as food in most cases and hence this discussion is mostly with respect to the muscle tissue. Significant variations may be observed in other cases. The basic constituents that make up the fish tissues are the same as those found in other animal tissues. Water is the major constituent, which accounts for 60-80% of the fresh tissue. Protein, fat (or lipid) and minerals are the other principal constituents. These four constituents together form about 95-98% of the total weight of the tissue. Carbohydrate is not a major component, especially in the muscle tissue. Small quantities of glycogen (usually about 0.5%) are present in almost all species. A variety of many other minor constituents like water-

soluble vitamins, free amino acids and nucleotides are also present in fish. Even though these are quantitatively less significant, each one has specific biological functions and the absence of these will lead to metabolic and physiological disorders. Therefore each component, irrespective of its quantitative abundance, is important for the organisms.

### Variations in Biochemical Composition

As has been indicated earlier, the biochemical composition of fish varies widely and these variations are determined by many factors, some intrinsic and some external. Table 1 gives an indication about the extent of variations that can be expected in fish.

Table 1: Principal constituents (percentage) of fish muscle

Constituent	Minimum	Normal range	Maximum
Protein	6	16-21	28
Lipid	0.1	0.2-25	67
Carbohydrate		0.5	
Minerals		1.0-2.0	
Water	28	65-80	90

The minimum and maximum values listed are the extremes and only rarely encountered. But variations are considerable even in the normal range, especially for lipids and to some extent, water.

### **Factors influencing biochemical composition of fish.**

Conditions like genetic characteristics, biological status in the life cycle, feeding habits, environmental conditions, etc. are the important parameters determining the chemical composition. The basic biochemical nature and the biological processes during spawning, migration etc. are controlled by genetic nature of the animal while availability of food and other environmental conditions are external factors which can influence the biochemical composition.

Migration and spawning of fish are energy-intensive processes and most species usually do not ingest much food during spawning and migration. This results in depletion of energy stored in the body. Fish having energy reserve in the form of fat will utilize this and in cases where the fat reserves are depleted and insufficient, protein is used as source of energy. The net result is a tissue depleted in terms of fat and protein contents. Once the spawning process is over and the fish starts normal feed intake, the rebuilding process starts. Protein content increases first, followed by fat content, till the normal status for the species is attained. The non-availability of feed due to any reason also will affect the biochemical composition of fish in a similar manner.

Lipid or fat is the component affected most by the above processes. Extreme variations can be observed in many species. A typical example is oil sardine (*Sardinella longiceps*) in which case the lipid content varies from about 3-4% in June-July to about 18% in December. Similar variations have been reported for many other species also.

In many species lipid is stored in organs like liver and in such cases, the lipid content of the muscle will be comparatively low and not prone to wide variations. Those species with low lipid content in the muscle are called lean fish. The site of lipid storage in several species is the muscle tissue and in such cases the muscle tissue will have high lipid content and also significant variations will take place in the lipid content under the influence of various factors. The species with high lipid content are classified as fatty species. It may however be noted that this classification is meant to give a general idea about the fat content of the tissue and is not well defined definition to distinguish these groups.

The distribution of lipids may not be uniform through out the body of the fish. Liver, which is the storage site for lipid in many species and in these cases the lipid content of liver will be much higher than that of other parts of the body. In certain species, lipid storage is in the subcutaneous layer and in some other, in the belly flaps. A gradual increase in the lipid content of muscle tissue from head to tail has been reported in many cases. Similarly, lipid content of white muscle and dark muscle differ significantly; red muscle being richer in lipids than the light muscle. It is also important to note that all these quantitative differences may be associated with qualitative differences as well.

Synthesis and accumulation of protein are not subject to the kind of variations found in the case of lipids. External factors have minimum influence on the protein levels and only extreme conditions result in any significant changes in most cases. Therefore, protein content of the muscle tissue is fairly stable when compared with

lipid content. However, there are instances of decrease in protein content, especially associated with spawning.

Water content of the tissue normally is in the range of 65-80%. This undergoes notable variations, particularly, when the lipid content is affected. There is an inverse relationship between lipid and water contents and as a general rule, the two together make up about 80% of the weight of the tissue. Thus, when there is a drastic reduction in the level of lipids, there is a proportional increase in the water content of the tissue.

The extent of variations in the concentrations of the quantitatively minor components like vitamins, non-protein nitrogenous compounds, carbohydrates etc. are not well understood and documented.

The analysis of the four major constituents of fish muscle viz., water, protein, fat and minerals (ash) is often referred to as 'proximate analysis'. Data on proximate composition are critical for several applications. These data are important for the food technologists, nutritionist, dieticians etc. But in spite of investigations on these lines from as early as 1880s, reliable data on proximate composition are not available for a large number of commercially important species. The main reason for this is the complex variations in the proximate composition due to so many variables. Sustained and systematic investigations are essential for accurate data on proximate composition.

### Nature and Functions of the Major Biochemical Constituents of Fish Muscle.

#### Water

Water is essential for all biological functions in fish. All metabolic processes

are taking place in aqueous media and major component of all biological fluids is water. Transport of metabolites, nutrients etc. depend entirely on these fluids. Another important function of water is in maintaining the native structure of protein molecules. Part of the water present in the tissue is in the form of 'bound water' and part is present in the free form, particularly, in the fluids. Any loss of water during processing and storage will affect the quality, especially the texture due to denaturation of proteins. Loss of water may result in loss of flavour also, because many of the flavour bearing compounds are water-soluble.

#### Protein

Proteins are high molecular weight polymers made up of amino acids. There are 20 naturally occurring amino acids and different permutations and combinations and varying degrees of polymerization can result in large variety of proteins having different properties and functions. Amino acids combine through peptide bonds and long peptide chains can be formed. This sequence of amino acids in the peptide chain is the primary structure of proteins.

The peptide chain is not remaining as a linear one. Due to the molecular geometry

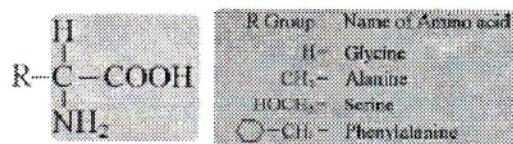


Fig. 1 Structure of amino acids

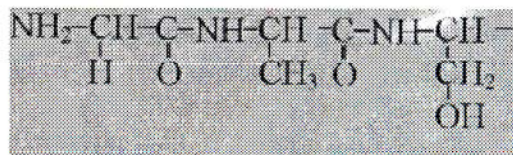


Fig 2 Peptide chain

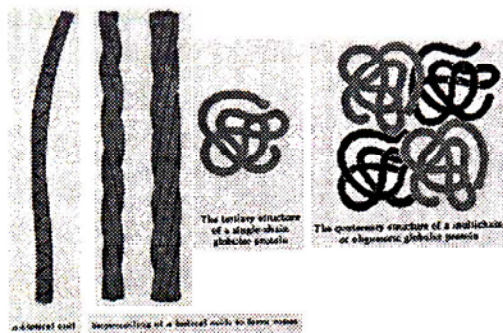


Fig. 3 Helical and globular structure of proteins and interactions of the various groups it gets folded, mostly into  $\alpha$ -helical form and maintained in the form through hydrogen bonding. These helical structures further fold into globular form and this form is retained through a variety of bonds like hydrogen bonds, disulphide bonds etc. The functional form of proteins is usually an aggregate of several such units. Retention of the natural configuration is a prerequisite for the proper biological function of these molecules. Changes in pH, temperature, ionic strength etc. affect the natural configuration and the proteins get denatured, often irreversibly, depending on the severity of the treatments.

#### Classification of fish muscle proteins

Based on solubility and functional characteristics, fish muscle proteins can be classified into three groups viz. sarcoplasmic, myofibrillar and stroma proteins.

##### *Sarcoplasmic proteins*

The fraction of protein that is soluble in neutral salt solutions of low ionic strength ( $<0.15M$ ) is classified as sarcoplasmic proteins. This fraction constitutes about 20-30% of total proteins and the major constituents are myoalbumin, globulin and enzymes. Composition of the enzymes present in sarcoplasmic fraction depends on the condition of the fish. Freezing, storage

etc. will result in damage to organelles and this will release enzymes localized inside endoplasmic reticulum, mitochondria and lysosomes. Therefore it had been suggested that the enzymes present in sarcoplasmic fraction can be an indicator of freshness of fish. However, this technique should be used with much caution because of the highly complex nature of the interaction involved in the process.

Detailed analysis of the myogen fraction of sarcoplasmic protein by electrophoresis has shown that the protein pattern of muscle myogen can be used for species identification. Different species have their characteristic pattern and this is not affected to any significant extent by nutritional status or growth. Therefore this has been accepted as a reliable technique for identification of species in a large number of cases.

##### *Myofibrillar proteins*

Proteins soluble in neutral salt solutions of fairly high ionic strength (0.5M) are classified as myofibrillar proteins. These are the structural proteins and include actin, myosin, tropomyosin, actomyosin etc. In fish, the proportion of myofibrillar protein is about 65-75% of the total protein (compared to about 40% in mammals). Myofibrillar proteins are responsible for the muscle movement.

Myofibrillar proteins are the most important from processing and product development angles. They are responsible for the functional properties like texture, water-holding capacity, gel forming capacity and fibrous nature. These proteins are denatured more easily than the other groups and this denaturation affects all the functional properties, leading to deterioration of quality.

The special qualities of myofibrillar proteins are utilized in development of special products like surimi. After removal of sarcoplasmic proteins by washing, the myofibrillar fraction is treated with salt and stabilizers. This results in partial denaturation and formation of strong gel, which can be processed further to various value added products.

#### ***Stroma proteins (connective tissue proteins)***

The third group is the fraction of protein that is not soluble in salt solutions or even in dilute acids or alkalis. Proteins like collagen and elastin, found mainly in the connective tissues, belong to this group. The proportion of stroma proteins is about 3% of total proteins in teleost fish and 10% in elasmobranchs, compared to about 17% in mammals. The soft texture of fish has been attributed to the low content of connective tissue. Collagen from fish muscle is much more thermo labile and contains fewer cross-links than collagen from warm-blooded vertebrates. Chemically, the high proportion of hydroxy proline distinguishes collagen from other types of proteins.

#### ***Proteins with special qualities***

Fish contains several proteins with unique properties. Highly basic proteins, protamines, found in the milt of some species of male fish are one such example. It is a low molecular weight protein and contains about 65% arginine. Another such protein is the antifreeze proteins found in many cold-water species. These can lower the freezing point and thus play an important role in survival of the fish under cold conditions.

#### **Amino acid composition of fish protein**

Fish proteins are made of  $\alpha$ - amino acids and all the 20 naturally occurring amino acids are found in varying proportions. Fish protein contains all the essential amino acids and this is one reason why fish is considered as a highly nutritious item for man.

#### **Fish protein in human nutrition**

Fish is an important source of protein and it is comparatively less costly also. It is easily digestible and has got high biological value compared to proteins from many other sources. It contains all the essential amino acids. All these factors make fish an ideal source of protein.

Apart from this, fish protein has some unique nutritional characteristics. It is now established that fish protein behaves as a hypocholesterolemic agent, probably because of the characteristic amino acid composition. Another recent finding is that fish protein is a strong cardio protective agent and can control the symptoms of experimentally induced myocardial infarction in albino rats. It protects the antioxidant system and thus minimizes the chances of tissue damage caused by oxidative stress.

#### **Lipids**

Lipids are defined as the fraction of any biological material extractable with a solvent of low polarity. This definition is not a precise one and is of a very general nature. A wide variety of compounds are included in this, making it a highly heterogeneous group. Any material extracted with 'fat solvents' like ethyl alcohol, ether, chloroform, hexane and petroleum ether can be described as lipid. The important types of compounds included

in this group are glycerides, phosphoglycerides, sphingolipids, aliphatic alcohols and waxes, steroids and combinations of the above types of compound with proteins, peptides, carbohydrates etc.

The most important types of compounds present in fish lipids are those derived from glycerol, a trihydric alcohol and fatty acids. When all the three -OH groups of glycerol are esterified with fatty acids, a triglyceride or triacylglycerol is formed. The three fatty acids esterifying with glycerol molecule may be the same, or different; the latter being the case in most naturally occurring lipids. Triacylglycerol is the major component in tissue lipids, especially when the fat content is high. Increase or decrease of fat content in the tissue is affecting the proportion of triacylglycerols directly while other types of lipids are affected to a much less extent. This is because of the fact that triacylglycerol is the preferred form of stored fat, except in certain special cases where hydrocarbons, wax esters etc. take the place of triacylglycerol.

When only one or two hydroxyl groups

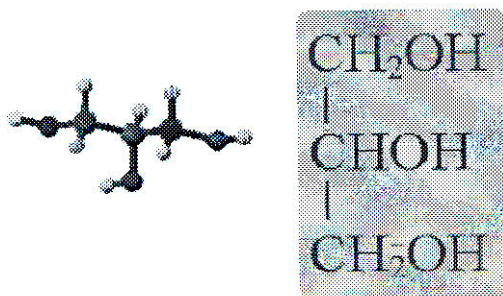


Fig. 4 Glycerol

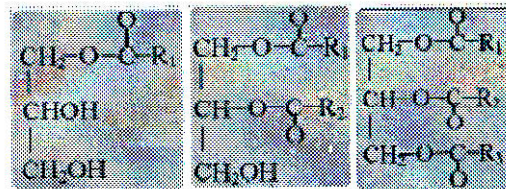


Fig. 5 A. Monoacylglycerol B. Diacylglycerol C. Triacylglycerol

of the glycerol are esterified with fatty acids, leaving the other hydroxyl groups free, monoacylglycerols and diacylglycerols are formed. Small quantities of these are present in fish lipids. These are mostly metabolic intermediates and in normal conditions, the proportion of these compounds will be very low.

Another important glyceride lipid is phospholipid. In phospholipids, hydroxyl groups at position 1 and 2 are esterified with fatty acids and the -OH group at position 3 will be combined with a phosphate group. Phosphatidic acid is the example of simplest phospholipids. The phosphate group combines with bases like choline ethanolamine, or serine, forming different phospholipids like phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine. The above three are the major phospholipids commonly present in fish tissue.

Phospholipids are major components of cell membranes. In association with protein, carbohydrates etc. phospholipids control the nature and function of cell membranes. Because of the structural function of phospholipids, they are called structural lipids. Normally, the proportion of phospholipids is about 0.5 to 1% of fish tissue. The proportion of phospholipids in the tissues does not undergo wide fluctuations as in the case of triacylglycerols.

There are many other compounds belonging to the lipid group, which do not have a glycerol backbone. Examples are waxes, steroids, sphingolipids, hydrocarbons etc. They are, in general described as non-glyceride lipids. Significant quantities of these compounds are present in the lipids of certain species of fish. Squalene, a hydrocarbon, is the major constituent of the liver oil of some deep-sea sharks. Wax esters and alkoxy diglycerides are some of the other types of compounds found in the lipids of certain species.

#### **Fat soluble vitamins**

Vitamins A, D, E and K are present in almost all fish lipids to varying extent and they are very important in controlling critical metabolic processes. Some of these vitamins are present in large proportion in certain fishes. Vitamin A in shark liver oil, and vitamin D in cod liver oil are typical examples. These oils can be used as vitamin supplements. Proportions of vitamin A and D are usually higher in liver when compared with muscle tissue. Fish is a good source of vitamin E, which is present as  $\alpha$ -tocopherol, the most potent among the various forms of the vitamin.

#### **Fatty acids**

Fatty acids (higher homologues of monocarboxylic acids) are present in most forms of lipids and the properties of the fatty acids play a very important role in determining the properties and functions of the lipids. Fatty acids present in fish lipids are unique in many ways.

One characteristic of fish fatty acids is that they are long chain fatty acids i.e. the number of carbon atoms in a molecule of fatty acid will be higher than that found in

fatty acids from other sources. Fatty acids with 12 to 22 carbon atoms are commonly present in fish lipids, and the most abundant members have 16 or more carbon atoms. Fatty acids of plant or other animal origin have shorter carbon chains.

Another important characteristic is the degree of unsaturation of the fatty acid molecule. Both saturated and unsaturated fatty acids are present in lipids, but uniqueness of fish lipids is the very high degree of unsaturation. Fish lipids contain saturated, monounsaturated (one double bond per molecule) and polyunsaturated (two or more double bonds per molecule) fatty acids. It is this group of polyunsaturated fatty acids, often referred as PUFA, that imparts fish lipids its unique properties.

Fatty acids with upto six double bonds are commonly present in fish lipids where as in lipids of plant or other animal origin, the number of double bonds per molecule of fatty acid is limited to 3 or in some cases, 4. This high degree of unsaturation of fish lipids is of great significance from nutritional and technological view points. Proportions of PUFA in fish body lipids is about 20-25%, 70-80% of which is accounted for by fatty acids with 4, 5 and 6 double bonds and, carbon chain length of 20 and 22 carbon atoms. Thus the most important feature of fish body lipids is the high proportion of long chain polyunsaturated fatty acids.

Another very important aspect about the fatty acids present in fish lipids is that the PUFA belong to the n-3 or  $\omega$ -3 groups, which are nutritionally very important for man. Recent researches have established that n-3 PUFA are highly beneficial in controlling a variety of disease conditions

like hypercholesterolemia, coronary heart diseases, cancer, rheumatoid arthritis and other inflammatory conditions, mental disorders etc. The n-3 PUFA are precursors to a group of compounds called eicosanoids, which control biochemical processes involved in a host of basic physiological functions. There is no other known source that can provide these n-3 PUFAs in such abundance.

At the same time, one has to take note of the challenges a food technologist will have to overcome while handling a commodity with such a high proportion of highly unsaturated fatty acids. These are highly susceptible to oxidation even at low temperatures and products of oxidation are highly undesirable in any food material. They adversely affect the flavour and odour (rancidity) and make the product unacceptable. A more important aspect is that the products of oxidation (peroxides, hydroperoxides) are toxic and even carcinogenic. So it is extremely important that formation of such products are prevented during processing and storage. Proper environmental conditions are to be maintained so that formation of these deleterious entities is minimized. Minimum exposure to oxygen, metal ions like Cu, Fe and elevated temperatures will help in minimizing the damage due to oxidation. The process of oxidation is self-sustaining, (called autoxidation) and therefore the most important point is to ensure that conditions, which initiate oxidation, are brought under strict control.

### Minerals

Several minerals are present in fish tissue and the major ones are sodium, potassium, calcium and phosphorous. Availability of iodine and selenium in

marine fish is of special significance from nutritional point of view.

Mineral composition of fish muscle (mg/100g)

Element	Average	Range
Sodium	72	30-134
Potassium	278	19-502
Calcium	79	19-881
Magnesium	38	5-452
Phosphorous	190	68-550

### Vitamins

Vitamin A, D, E and K, the fat-soluble vitamins, are present in lipids. Water soluble vitamins like Vitamin B are present in appreciable quantities in fish. Vitamin content of fish in general is comparable to that of mammals except in the case of vitamin A and vitamin D.

### Non-protein nitrogenous compounds

Fish muscle contains a group of compounds containing nitrogen, but these are not proteins. These are low molecular weight compounds and are present in the water soluble fraction. A number of compounds are present in this group and generally they constitute 9-18% of the total nitrogen in teleosts. The proportion will be higher in elasmobranchs. Major compounds present in this group are free amino acids, creatine, trimethylamine oxide, urea, nucleotides etc. The proportion of the individual compounds varies with species and also within species, based on size, season etc. Urea content in elasmobranchs is higher than that in teleosts. Similarly, trimethylamine oxide (TMAO) is present in muscle tissue of marine species, but is usually absent in fresh water species. The NPN fraction also contains free amino acids. Taurine, alanine, glycine and

imidazole-containing amino acids dominate the NPN fraction in most fish. Proportion of histidine, an imidazole containing amino acid in scombroid fish is of special interest as far as the quality of stored fish is concerned. Decarboxylation of histidine results in production of histamine, a toxic substance and high levels of histamine has been found to be dangerous.

The origin and role of these NPN compounds in fish have not been well understood. Many of these compounds have important metabolic and physiological functions. High-energy molecules like nucleotide phosphates are used for transport of energy. Free amino acids and TMAO are involved in osmoregulation. Some of these compounds are intermediates of metabolic processes.