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Lecture Notes

Part 1

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CHANGES IN BIOCHEMICAL COMPOSITION OF FISH

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Introduction

Fish 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. 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 constituents viz., carbohydrates, proteins, fats and minerals and vitamins in correct proportion. In this modern era of health conscientious people, presence of one component or other like cholesterol is not appreciated in the diet. In this context, fish assumes significance and fish caters to the needs of the consumers besides contributing significantly to nutrition. No doubt 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 composition (Baldwin, 1951). The composition of the fish depends also on the season, habitat and the type of food consumed by the fish.

Moisture, fat, protein and minerals are the main components of fish meat and the analysis of the same is referred to as ‘proximate composition’ (Love, 1970). 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.

Composition

Like any other meat, the water, protein, lipid and ash (mainly minerals) constitute the four basic constituents of fish meat (Tables 1 and 2). 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.

Table 1. Proximate composition of edible portion of fish

Moisture	65.0 –90.0 %
Protein	16.0 – 21.0 %
Fat	0.2 – 25.0 %
Ash	0.5 – 5.0 %

Water

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 contents. Low water content is usually associated with relatively high fat content and vice versa. An example of a species with high water content is bombayduck (*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 viz. sarcoplasmic proteins, myofibrillar protein and stroma. Sarcoplasmic proteins (albumin and globulin) are soluble in 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 (flatfishes, 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 myofibrillar proteins are (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 approximately the same as for the corresponding proteins in mammalian muscle, although the physical properties may be slightly different. 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. Some of these changes have beneficial effects in the processing of fish for product development. 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.

Connective tissue proteins (also called stroma proteins) constitute approximately 3% of the protein in teleosts and about 10% in elasmobranchs (compared with 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 and is very similar to that present 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 and may be related to the swimming behaviour. Further, the varying amounts and types of collagen in different fishes may indicate the differences in 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 3). 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.

Lipids

Lipids are heterogeneous group of compounds extracted with solvents of low polarity. Glycerides, fatty acids, phosphoglycerides, sphingolipids, aliphatic alcohol and waxes, steroids and several lipoproteins are the important components belonging to this group. The terms fat or oil are often used to represent this group of compounds.

In terms of quantity, lipid is the third major constituent in fish muscle. Variation in the fat content is much wider than that of protein. Fat content varies between species, and also between different organs within species. Fish with fat content as low as 0.5% and as high as 18-20% are common. In many species there is a build up of fat during feeding season and its proportion decreases substantially after spawning. As far as the type of lipid in fish muscle is concerned, triacyl glycerol and phosphoglycerides both containing long chain fatty acids are the major components. Squalene and wax esters are other components found in unusually high concentrations in a few fish meat.

Some tropical fish also show a marked seasonal variation in fat content. The west African shad *(Ethmalosa dorsalis)* has 2-7 % (wet weight) fat content over the year with a maximum in July. Similarly the Indian oil sardine (*Sardinella longiceps*) show high variations in fat content and during seasons the content go up to 18%. Based on these variations in fat content, fish are broadly classed as lean (fish that store lipids only to a limited extent) and fatty fishes (fish storing lipids in fat cells distributed in the body tissues). Typical lean species are the bottom-dwelling ground fish like cod, saithe and hake. Fatty species include the pelagics like sardines, herring, mackerel and sprat. The fat is the source of stored energy. The storage sites for fat (fat depot) are different between species. Liver, adipose tissues etc. are the common sites for fat storage in fish. In a great majority of cases, the depot fat is mainly triglycerides.

The white muscle of a typical lean fish (cod) contains less than 1% lipids. The lipids present in a teleost fish species may be divided into two major groups viz., the phospholipids and triglycerides. The phospholipids (about 0.7-0.8% of the tissue) make up the integral structure of the membranes in the cells and are often called structural lipids. Phosphatidyl-choline (70%), phosphatidyl-ethanolamine (19%) and phosphatidyl-serine (5%) constitute the major phospholipids though there are other types in minor quantities. The triglycerides are lipids used for storage of energy in fat depots and are often termed depot fat. Besides the phospholipids the membranes also contain small quantity of cholesterol, which contribute to the rigidity of the membrane. The adipose tissues making up the lipid depots in fatty species are typically located in the subcutaneous tissue, in the belly flap muscle and in the muscles moving the fins and tail.

Lipids of fish and other aquatic animals contain high proportion of highly unsaturated long chain fatty acids. Fatty acids with carbon chain varying from 10 to 22 and unsaturation varying from 0 – 6 double bonds are common. Among the saturated acids palmitic and stearic acids are the important ones and in the monounsaturated group, palmitoleic and oleic acids are the major constituents. Among the polyunsaturated acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the major components. The percentage of polyunsaturated fatty acids with four, five or six double bonds is slightly lower in the lipids from freshwater fish than in the lipids from marine fish. However, the composition of the lipids can vary with the food intake and season and many other factors.

The biological significance of fish oils is mainly due to the presence of polyunsaturated fatty acids. In human nutrition fatty acids such as linoleic and linolenic acid and arachidonic acid are regarded as essential since they cannot be synthesized in the system. In marine fish, these fatty acids are present at a relatively low level. However, presence of other polyunsaturated fatty acids gives importance to fish oils compared to vegetable oils. These acids, eicosapentaenoic acid (C20:5 w3) and decosahexaenoic acid (22:6 w3) are reported to play an important role in the development of nerve cells in growing children, besides having proven effects in atherosclerosis and as antithrombotic factor.

Fish oils have a positive cholesterol lowering effect by increasing the content of high density lipoprotein and lowering the LDL/HDL ratio. Evidences indicate the positive effect of PUFAs to reduce cardiac arrhythmias - the irregular electrical conductivity of heart muscle often leading to cardiac arrest. Presence of high levels of PUFA increases the membrane permeability and has an effect on blood pressure.

Approximately 50% of fatty acids in lean fish and 25% of fatty acids in fatty fish are PUFAs. Eicosapentaenoic acid (C20:5 w3) and decosahexaenoic acid (22:6 w3) are the major PUFAs associated with fish oils. EPA and DHA are reported to reduce vasoconstrictor effect by competing with arachidonic acid, which results in strong vasoconstriction through the formation of thromboxane A2, a strong vasoconstrictor. EPA is also reported to have antitumor effect through suppression of cell proliferation while DHA appears to induce apoptosis. Other clinical symptoms where PUFA plays key role include acute respiratory distress syndrome, multiple sclerosis, Alzheimer's diseases etc.

The highly unsaturated nature of fish lipids also creates problems in processing and preservation of fish. They are highly susceptible to oxidation on exposure to air, even at ambient temperature, leading to oxidative rancidity. The products of oxidation impart off flavour to fish and fish products and some of the oxidized products are even toxic. The polyunsaturated fatty acids are prone for oxidation leading to total unacceptability of fish or fish product. The other type of problem is the development of hydrolytic rancidity leading to the formation of free fatty acids. These free fatty acids react with proteins causing protein denaturation.

In elasmobranchs, such as sharks, a significant quantity of lipid is stored in the liver and may consist of high molecular weight hydrocarbon like squalene. Some sharks may have liver oils with a high concentration (80%) of the lipid as unsaponifiable substance, mostly in the form of squalene.

Minerals

Fish is a good source of almost all the minerals present in seawater (Table 5) and the total mineral content in wet fish meat ranges from 0.6 to 1.5%. Calcium and phosphorus account for more than 75% of the mineral in the skeleton. Besides forming a part of skeleton, phosphorous has a number of metabolic and physiological roles in fish. Elements of special nutritional significance such as iodine and fluorides are also present in fish. The iodine content in marine fishes ranges form 0-300 ìg per kg fish. Sulfur is present in the form of amino acids as fish is a good source of sulfur containing amino acids, cysteine and methionine. Copper and iron are associated with muscle tissues. Cobalt is present in the form of cyanocobalamin (Vitamin B_{12}). It should be noted that the sodium content of fish meat is relatively low which makes it suitable for low-sodium diets. Certain fishes like tuna are especially good sources of macro minerals like magnesium and trace elements like selenium.

Vitamins

Both water soluble and fat-soluble vitamins are present in fish. The hepatic reserve of Vitamin A in aquatic animals is much greater compared to mammals and birds. The meat of fatty or semi-fatty fishes is an excellent source of vitamin D, the concentration of which varies from 500 to 3000 IU/100g. In the meat, vitamin E occurs largely as a-tocopherol. The antihaemorrhage factor, vitamin K is also present in fish.

Fish meat is a good source of B vitamins. The red meat has higher content of vitamin B than the white meat. Fish liver, eggs, milt and skin are good sources of B1, riboflavin, pyridoxine, folic acid, biotin and B12. The amount of vitamins and minerals is species-specific and can vary with season. The vitamin content is comparable to that of mammals except in the case of A and D vitamins, which are found in large amounts in the meat of fatty species and in abundance in the liver of species such as cod and halibut. Liver oils from shark (13300 IU/ g oil) and tuna are rich in vitamin A. Large quantity of vitamin E, varying between 500 – 3000 IU per 100 g, is present in liver and body oils.

Non Protein Nitrogenous compounds

These water-soluble, low molecular weight, nitrogen-containing compounds of non-protein nature constitute 9 to 18% of the total nitrogen in the teleosts. The major components in this fraction are: volatile bases such as ammonia and trimethylamine oxide (TMAO), creatine, free amino acids, nucleotides and purine bases, and, in the case of cartilaginous fish, urea.

Quantitatively, the main component of the NPN-fraction is creatine. In resting fish, most of the creatine is phosphorylated and supplies energy for muscular contraction. The NPN-fraction also contains a fair amount of free amino acids. Taurine, alanine, glycine and imidazole-containing amino acids seem to dominate in most fish. Of the imidazole-containing amino acids, histidine has attracted much attention because it can be decarboxylated microbiologically to histamine. Active, dark-fleshed species such as tuna and mackerel have a high content of histidine.

Changes in Biochemical composition

Changes or alterations in biochemical composition take place due to various reasons viz., the natural conditions, spoilage and processing which in turn depends on the type and quality of the fish in question.

Natural

Under natural conditions, numerous fish species endure long periods of starvation associated mainly with seasonal changes in food availability, spawning migrations, preparation for spawning or seasonal changes in water temperature. These are changes taking place in fish in the normal process and results in subtle changes in the composition. There are changes in the lipid fraction during these conditions primarily due to conversion of lipids for production of energy. Relatively little changes is noticed in the protein fractions initially but excessive starvation affects protein content as well.

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 ETC and oxidative phosphorylation mechanisms are no longer operative. This results in the depletion of ATP and creatine phosphate. Along side 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. A prominent postmortem change is the loss of water. This is related to the drop in pH to 5.3 – 5.5 which is almost close to the isoelectric pH of fish meat, which in turn is related to the decrease in ATP level. During post rigor aging of meat, the water holding capacity of meat was found to increase. This is attributed to an increased osmotic pressure within the fibres or alterations of the electrical charges on protein molecules involved, and is related to the movement of ions to and from the muscles.

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 with appropriate functionalities.