

Proximate composition of fish

The proximate composition of most fish and shellfish is primarily water, proteins and lipids and these constituents make up about 98% of the total mass. The other minor constituents include carbohydrates, vitamins and minerals. Proximate data on different fish species are collected in databases such as the FishBase (www.fishbase.org). The chemical composition of fish varies due to seasons, geographical locations, stages of maturity, and sizes.

Marine lipids contain the omega-3 fatty acids, such as C20:5 n-3 (EPA) and C22:6 n-3 (DHA) with well documented beneficial health effects. These fatty acids are found in all parts of the fish. They are constituents of different lipid classes such as phospholipids, triacylglycerols, lysophospholipids, partial glycerides, esters and free fatty acids. Marine lipids are the only source of EPA and DHA, and extraction and utilization of these fatty acids is a major industry. The market shares for higher value applications such as food ingredients, health care products and medicine are increasing owing to the supply to aquaculture business.

Fish protein is regarded to be of high nutritive value due to its favourable content and balance of essential and non-essential amino acids. Seafood proteins are also highly digestible, with digestibility of raw fish meat in the range of 90-98% and that of shellfish about 85%. Protein and amino acid requirements vary through life and are generally higher among young growing children compared with adults. Fish and marine invertebrate tissue contains from about 11-24% crude protein depending on species, nutritional conditions and the type of muscle. Although amino acid composition might vary among different type of tissue, there is a high similarity in the same tissue among species. The total body composition of amino acids shows high similarity among various cultured fish species.

Carbohydrates are often classified into three broad groups: sugars (mono and disaccharides), oligosaccharides (3 to 9 monosaccharides) and polysaccharides (> 9 monosaccharides). The content of carbohydrate in fish muscle is low and is further influenced by conditions experienced before and during capture, which may lead to depletion of glycogen stores and thereby decrease in the carbohydrate level. Under anoxic conditions postmortem, glycogen will continue to be metabolized, resulting in increased lactic acid along with reduced pH and eventually a gradual loss of the sweet, meaty character of fresh fish. Some marine invertebrates, on the other hand are characterized by a high content of carbohydrates; up to 10.2% and 12.5% total sugars can be found in subcuticular tissue of spiny lobster and blue crab, respectively, with the highest amounts of glucose followed by galactose and mannose. Glycogen stores of scallops are highly dependent on season such a temperature, food availability and life cycle. Highest levels are usually reached after the summer period, showing levels up to 23-25% glycogen in dry weight of adductor muscle. Seasonal variations of glycogen content in mussels are also high, showing values in the range of 4-37% of tissue dry weight.

The energy content of food is generally given in kilocalories and kilojoules, which have a conversion factor of $1 \text{ kcal} = 4.184 \text{ kJ}$. Seafood show variable composition of proteins and fat, and energy content is dependent on this distribution, which is highly influenced by seasonal variations. In a seasonal variation, even mackerel known as fatty fish could be classified within the lean fish. The lipid level in particular has high significance for the calorie content of fish, with implications for calculations in dietary studies and databases.

Methods of analysis

There are several methods to analyze the major components in seafood. Analysis of each nutrient separately is time-consuming and requires a diverse set of equipments. Methods for simultaneous determination of the major components are therefore valuable.

1. Near infrared spectroscopy (NIR) is the most common method for such analysis. NIR is found to be reliable, rapid and easy to perform nondestructive analysis for simultaneous determination of the major components of fish. The development of NIR in food analysis started with the development of analysis of cereal grains and oilseeds in Canada. It has resulted in a total cost saving of Canadian dollars 2.5 m per year and saving for the environment by replacing the kjeldahl system, which involves concentrated sulfuric acid and heavy metal catalysts and reducing the caustic wastes. NIR is thus a chemical free method.

The first instruments in the market were filter instruments measuring the reflectance mode. Later, in 1980's, monochromators instruments were developed to measure the whole spectrum. The NIR spectrum is defined between the wavelength 800 and 2500 nm. The available detectors cover a smaller range. Silicon detector covers the range 400-1100 nm, an indium gallium arsenic covers the range 800-1700 nm, and a lead sulfide covers the range 1100-2500 nm. The NIR radiation interacting with a sample may be absorbed, transmitted or reflected depending on the interaction with NIR wavelength. The physical status of the sample is transparent or non transparent. Diffuse transmittance measurements are usually carried out in the 800-1100 nm region of the spectrum, where the weak absorptions enable useful data to be obtained using sample thickness of 1-2 cm (meat, cheese or whole grain). In this spectral region, the **spectrum of the transmitted light is very compact** and no single peaks are visible, making it difficult to use this spectral range before the development of multivariate calibration technology, such as the introduction of **partial-least squares (PLS)**. The spectral data will be reduced by principal component analysis (PCA) and then one can perform a **linear regression** on the principal components. The end result is the **calibration equation** from which the constituent of interest is calculated from a linear combination of spectral data.

Near infrared transmittance (NIT) instruments are particularly suitable for the analysis of fish. The sample has to be minced, and it is usually possible to run several subsamples. The results are averaged to obtain more representative spectral data from the sample. The spectral data are then used to perform multivariate calibrations against the chemical and physical data. The same

spectral data will be used against the different selected variables, so one can simultaneously predict water, fat and protein content from the spectral data as accurately as the traditional chemical methods.

To analyze directly on a fillet one needs an interactance probe. This involves illumination and detection at laterally separated points on the sample's surface. It is normally accomplished using a fibre optic bundles carries the incident radiation and another carries the reflected radiation. It is necessary to have a large interactance probe usually two times 2 cm, due to the striped structure of fish muscle. It is possible to make analysis directly on the fillet with this probe without previous mincing but with a slightly lower accuracy.

Portable instruments are now available and successful results are also obtained for whole fish and for live fish. instead of a conventional monochromator, instruments are now also made with diode arrays, making it possible to measure the whole spectrum at the same time and in that way reducing the time for measurement, making online analysis possible.

NIR absorption will change with temperature and calibration. NIR measurements must therefore be made on samples with approximately the same temperature. The measurements are also affected by texture and whether the sample has been frozen and thawed. Due to the requirement of extensive sample specific calibrations, the analysis should be performed by skilled personnel. Once calibrated, the analysis is easy to perform.

2. Nuclear magnetic resonance (NMR) is another nondestructive technique that enables determination of fat and water. Recent studies have shown it is also possible to gain data on protein levels in the dried samples. The low field NMR instruments commonly in use require the withdrawal of cylindrical samples of 10-40 mm diameter for analysis. The method is fast, accurate and easy to use, when calibrations are performed. A handheld portable NMR instrument (NMR mouse) has recently been developed and it enables an analysis time of less than 20 sec and can even used in vivo on living fish.

3. Less common methods for nondestructive analysis of proximate composition in fish are ultrasound techniques, the total body electrical conductivity (TOBEC) techniques and magnetic resonance imaging (MRI). The ultrasound method is rapid, automated and can be used online and empirical equations have been developed to relate the ultrasonic velocity to composition. A weakness in this method is the variations in the ultrasonic properties of fish tissue due to temperature. For nonfatty fish, the solid non fat content can be determined from a single measurement, however at least two temperatures are suggested during analysis of fat and solid non fat in fatty tissue. In the TOBEC method, the live fish is placed in a low frequency electromagnetic field, and the distinct electrical characteristics of body fat and fat free tissue provide the proximate data. MRI can provide valuable information on proximate composition and distribution of chemical constituents of fish samples, however these imaging instruments are expensive and are used primarily in certain research laboratories.

Calculation of fat content by measuring the water content is possible with cheap, robust instruments but they can be used only when the protein content is stable.

Minerals and Trace Elements:

The total mean mineral content (ash) of fish fillet is very close to 1% of its wet weight. Some fatty fish species exceed this figure up to 1.6% (eg. *Sardine pilchardus*) and some mollusks, even higher, more than 2%.

The average values of aluminum in North sea fish varied between 0.05 – 0.28 mg/kg wet weight. The average overall value in species was close to 0.1 mg/kg wet weight. The value in species from open Northeast Atlantic waters varied between 0.03 – 0.14 mg/kg. The species from other areas such as Barents Seas, Greenland waters and Baltic sea exhibit similar concentration, never exceeding 0.2 mg/kg. Species from Norwegian waters showed elevated aluminum concentrations up to 0.94 mg/kg due to the presence of an aluminum smelter plant in that area. The marine fish from waters not affected by aluminum producing industry contains in its edible part approx. 0.1 mg/kg aluminum wet weight. Marine shellfish had generally high aluminum levels compared with fish species. There was a variation from 0.2 mg/kg in squid to 4.95 mg/kg in blue mussels from North sea. An analysis of different organs of cod showed quite different aluminum concentrations in body compartments. All being higher than that in edible part: 0.06 mg/kg in female gonads, 0.22 mg/kg in liver, 0.29 mg/kg in spleen, 0.36 mg/kg in kidney, 0.44 mg/kg in brain, 0.45 mg/kg in heart and 0.63 mg/kg in gills.

Arsenic concentration, As (V) and As (III) in the surface water of the world's ocean is close to **1 µg/L**. Arsenic taken up by marine animals is concentrated in their bodies by metabolic processes. It can be found in seafood in many different forms as inorganic arsenate or arsenite, as organic methyl arsonate and dimethyl arsonate, as arsenobetaine, arsenocholine, trimethyl arsine oxide and as arseno sugars. Human volunteers who consumed 100g plaice fillet containing 80mg arsenic demonstrated that organic arsenic was almost quantitatively excreted as trimethyl arsine by man. The organic forms of arsenic are non toxic to human, the **inorganic arsenic** is the **toxic form**. An inorganic arsenic proportions range from 0.07 - 0.1% of total arsenic concentration. Total arsenic concentration in species varied considerably between species (2-50 mg/kg wet weight), however the concentration of toxic inorganic arsenic remained low (about 0.1 mg/kg wet weight). In a variety of seafood samples including fish, crustaceans, bivalves and marine mammals, the levels of inorganic arsenic were below the **limit of detection** (0.0006 mg/kg wet weight). Arsenic in seafood does not pose any risk to human health because the major share components in seafood is **organic arsenic (>98%)** with a low toxicity.

Arsenic seems to be higher in **flatfish species** such as plaice and lemon sole, whereas gadoid species are generally low in arsenic. Different arsenic species, especially **arseno sugars** are detected in **marine algae**. Concentrations of four arseno sugars in four species of algae range from 1.05 – 10.04 mg/kg dry weight. IC-ICP-MS is used to determine arsenic, arsenate and

methylated and other organic forms of arsenic. HG-AAS and HG-AFS are used for total arsenic determinations.

Chromium levels in different species vary from 0.01 – 0.43 mg/kg wet weight with cod having the lowest and mussel having the highest. The average concentration of chromium in fish is 0.032 mg/kg wet weight. Chromium in the squid is 0.052 mg/kg wet weight. In the clam, it was found at levels ranging from 0.78 – 1.39 mg/kg wet weight depending on the season. The chromium content in seafood seems to vary considerably according to species and geographic origin between 0.01 mg – 0.5 mg/kg wet weight. Pacific oysters purchased from Hong kong markets had chromium content ranging from 1 mg/kg to > 35 mg/kg dry weight. The average chromium content in the cut trough shell was 0.47 mg/kg wet weight. GF-AAS is commonly used for chromium determination.

Iodine is an essential trace element for human beings required for normal activity of the thyroid hormones, thyroxine and triiodothyroxine. Seafood is the only natural source that contains a considerable amount of iodine. The iodine content in the skin of fish is much higher than that in the edible part. In saithe and cod fillet 0.68 and 0.29 mg/kg wet weight, while in saithe and cod skin 13.26 and 5.95 mg/kg wet weight. Considerable part of iodine is located in the lipids of fish (28% in mackerel, 45% in halibut, 68% in Atlantic salmon and 62% in ocean perch). Their concentration also varies within a given species eg. haddock 0.14 - 6 mg/kg. Lean fish had an average iodine content of 0.86 mg/kg wet weight, while fatty fish have 0.4 mg/kg wet weight in Norway. The average concentration of iodine in marine fish was 2.1 mg/kg and in freshwater fish 0.38 mg/kg in Switzerland. The iodine concentration in edible parts of Atlantic salmon during a feeding of iodine enriched feed was 1.4 mg/kg wet weight. Iodine is analyzed by ICP-MS nowadays. GC method can also be used.

Manganese content is 2.4 – 15.9 mg/kg in mussel, 4.9 mg/kg in clam, 5.8 mg/kg in tuna and 4.8 mg/kg dry matter in sardine. In wet weight, mussel had 0.22-0.30 mg/kg, clam 0.20 mg/kg and oyster .27 mg/kg. In shrimp species of Turkey, manganese was found at 0.25 – 1.52mg/kg wet weight. In Indian waters, manganese concentration range from 11.2 – 15.2 mg/kg wet weight in shrimps. Edible part of octopus of Portuguese coast had 1.5 – 2.39 mg/kg wet weight. Manganese determination is made by flame AAS.

Molybdenum amount of 3.74 mg/kg was reported in Chinese shrimps using a spectrophotometric method.

Selenium is a trace element like iodine as an essential nutrient of fundamental importance in human biology. Seafood is the major natural source of this element. Bioavailability of selenium from fish was high for humans and rats. A proportion of 23-34% of total selenium from fish was found to be soluble selenium. There is no big variation in selenium content of fish and it range from 0.1 to 1 mg/kg, with an average selenium content of about 0.4 mg/kg. Selenium content in

eight Indian fish species amounted to 4.4 mg/kg dry weight. HG-AAS is used for selenium estimation.

Thallium content in seafood is rare. In fish muscle, it is 1 µg/kg, in dog fish 3.2 µg/kg, trout 110.5 µg/kg. In the marine species of Southeast Asia, thallium is present in concentration less than 0.001 – 0.038 mg/kg dry weight. Majority of fish have vanadium concentrations around 10-30 µg/kg. Vanadium analysed by LC-ICP-MS or electrothermal AAS.

Zinc is an essential element and zinc containing foods must be a part of the human diet. The average zinc content of fish ranged from 1.0 – 27.0 mg/kg. Eels have high average content of 21.7 mg/kg, followed by herring 7.18 mg/kg. In mussels 20.7 mg/kg, in shrimps 16.8 mg/kg, oyster 49.1 mg/kg. zinc content vary largely depending on fishing area, probably season and intrinsic factors such as state of maturity. Zinc in edible parts of octopuses of South Indian ocean were 113 -138 mg/kg dry weight. An average zinc content was 3.80 mg/kg wet weight. Zinc is determined by AAS.

Other elements; lithium content in some marine smoked fish was 0.55 mg/kg. In edible parts of fish, it is in the range of 0.002 – 0.11 mg/kg wet weight. The concentration of rubidium varies around 2.2 mg/kg wet weight. In freshwater fish the average rubidium concentration was at 20 mg/kg dry weight. Cobalt in fish species was generally low with a minimum of 0.002 mg/kg and a maximum of 0.008 mg/kg. Most concentration was close to 0.003 mg/kg with an exception, eel has 0.02 mg/kg wet weight. Bromine in fish muscle tissue was 33.4 mg/kg dry weight with low values in sardine (8.6) and albacore tuna (8.7) and high values in clam species (48-54). Nickel was reported to be 2.9-6.1 mg/kg dry weight in mullets.

The ratio of sodium: potassium was between 1:5 and 1:6.